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Tissue Mineral Imbalances in Cattle with Brisket Disease

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TISSUE MINERAL IMBALANCES IN CATTLE WITH BRISKET DISEASE

by

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	iii
ABSTRACT	x
INTRODUCTION	1
REVIEW OF LITERATURE	5
Brisket Disease: Description and Theories of Cause, Occurrence, and Control	5
Brisket Disease and Mineral Metabolism	8
Calcium	9
Chloride	14
Cobalt	15
Copper	17
Iron	20
Magnesium	22
Molybdenum	25
Phosphorus	27
Potassium	29
Sodium	31
Zinc	33
EXPERIMENTAL METHODS	35
Experimental Design	35
Experimental Animals	35
Executing the Design	35
Acquisition procedure	36
Procedure for slaughter and tissue collection	37
Hematologic Studies	39

TABLE OF CONTENTS (Continued)

	Page
Sample Preparation	39
Bone Density Determinations	42
Volume	42
Weight	42
Density	42
Percent Absolute Dry Matter and Percent Ash	43
Supplies	44
Labware	44
Washing procedure	44
Distilled water and reagents	45
Tissue Digestion for Chemical Analyses	45
Mineral Analyses	46
Methods	46
Dilutions	47
Standards	48
Standard curves	48
Sequence of processing and analyses	49
Extraction for Cobalt and Molybdenum	50
Liver	50
Whole blood	52
Data Transformations	53
Statistical Analyses	54
RESULTS	55
Results of the Ante- and Post-mortem Physical Examinations	55
The ante-mortem physical examination	55
Post-mortem findings	55
Results of Chemical Analyses	57
Tables of results	57
Changes associated with brisket disease	58
Changes associated with increased age	61
Changes associated with the age-disease interaction	64

TABLE OF CONTENTS (Continued)

Page

DISCUSSION

Determinations Other Than Tissue Chemistry . 65

Control Study 66

Chemical Changes Associated with Brisket Disease 68

Calcium 68
Chloride 70
Cobalt 71
Copper 72
Iron 73
Magnesium 74
Molybdenum 77
Phosphorus 78
Potassium 79
Sodium 81
Zinc 82
Percent dry and percent ash 83

Chemical Changes Associated with Increased Age 84

Calcium 84
Copper 84
Chloride 84
Iron 85
Magnesium 85
Phosphorus 85
Potassium 85
Sodium 86
Zinc 86
Other parameters 86

Chemical Changes Associated with the Age-Disease Interaction 86

SUMMARY 89

LITERATURE CITED 93

APPENDIX 100

VITA 188

LIST OF TABLES

Table		Page
1A.	Experimental animals	101
1B.	Selected organ weights and volumes of experimental animals	102
1C.	Summary of selected physical and biological parameters in experimental animals	103
2.	Chemical analyses performed, and dilutions of the sample or primary digest solution used for analysis	104
3.	Operating conditions of the atomic absorption spectrophotometer	105
4.	Standards for chemical determinations	106
5.	Group averages from Bailey (7)	107
6.	Calcium concentration in cardiac tissue	108
7.	Calcium/magnesium ratio in cardiac tissue	109
8.	Calcium/potassium ratio in cardiac tissue	110
9.	Calcium concentration in hepatic tissue	111
10.	Calcium concentration in renal tissue	112
11.	Ionic calcium concentration in blood serum	113
12.	Total calcium concentration in blood serum	114
13.	Calcium concentration in right metatarsal bone	115
14.	Calcium/phosphorus ratio in right metatarsal bone	116
15.	Chloride concentration in blood serum	117

LIST OF TABLES (Continued)

Table		Page
16.	Copper concentration in cardiac tissue	118
17.	Copper concentration in hepatic tissue	119
18.	Copper concentration in blood serum	120
19.	Iron concentration in cardiac tissue	121
20.	Iron concentration in hepatic tissue	122
21.	Iron concentration in whole blood	123
22.	Magnesium concentration in cardiac tissue	124
23.	Magnesium concentration in hepatic tissue	125
24.	Magnesium concentration in renal tissue	126
25.	Magnesium concentration in blood serum	127
26.	Magnesium concentration in right metatarsal bone	128
27.	Phosphorus concentration in cardiac tissue	129
28.	Phosphorus concentration in hepatic tissue	130
29.	Phosphorus concentration in renal tissue	131
30.	Phosphorus concentration in blood serum	132
31.	Phosphorus concentration in right metatarsal bone	133
32.	Potassium concentration in cardiac tissue	134
33.	Potassium concentration in hepatic tissue	135
34.	Potassium concentration in renal tissue	136
35.	Potassium concentration in whole blood	137
36.	Potassium concentration in blood serum	138
37.	Potassium concentration in right metatarsal bone	139
38.	Sodium concentration in cardiac tissue	140

LIST OF TABLES (Continued)

Table		Page
39.	Sodium concentration in hepatic tissue	141
40.	Sodium concentration in renal tissue	142
41.	Sodium/potassium ratio in renal tissue	143
42.	Sodium concentration in whole blood	144
43.	Sodium concentration in blood serum	145
44.	Sodium concentration in right metatarsal bone	146
45.	Zinc concentration in cardaic tissue	147
46.	Zinc concentration in hepatic tissue	148
47.	Zinc concentration in blood serum	149
48.	Zinc concentration in right metatarsal bone	150
49.	Sum of minerals assayed in right metatarsal bone	151
50.	Percent absolute dry matter in cardiac tissue	152
51.	Percent absolute dry matter in hepatic tissue	153
52.	Percent absolute dry matter in renal tissue	154
53.	Percent absolute dry matter in defatted right metatarsal bone	155
54.	Percent ash in cardiac tissue	156
55.	Percent ash in hepatic tissue	157
56.	Percent ash in renal tissue	158
57.	Percent ash in defatted right metatarsal bone	159
58.	Percent ash in absolutely dry, defatted right metatarsal bone	160
59.	Summary of results in cardiac tissue	161
60.	Summary of results in hepatic tissue	162
61.	Summary of results in renal tissue	163

LIST OF TABLES (Continued)

Table		Page
62.	Summary of results in whole blood	164
63.	Summary of results in blood serum	165
64.	Summary of results in osseous tissue	166
65.	Summary of calcium results	167
66.	Summary of chloride and cobalt results	168
67.	Summary of copper and iron results	169
68.	Summary of magnesium results	170
69.	Summary of molybdenum results	171
70.	Summary of phosphorus results	172
71.	Summary of potassium results	173
72.	Summary of sodium results	174
73.	Summary of zinc results	175
74.	Summary of percent absolute dry matter results	176
75.	Summary of percent ash results	177
76.	Summary of the changes in tissue chemistry with brisket disease	178
77.	Summary of the changes in tissue chemistry with increased age	179
78.	Summary of the changes in tissue chemistry with the age-disease interaction	180
79.	Analyses of variance	181

ABSTRACT

Tissue Mineral Imbalances

in Cattle with

Brisket Disease

by

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Twenty four cattle, six each of healthy cows and calves, and cows and calves with brisket disease, were obtained, examined and slaughtered. The concentrations of calcium, chloride, cobalt, copper, iron, magnesium, molybdenum, phosphorus, potassium, sodium and zinc; and percent absolute dry matter and percent ash were determined in tissues selected from the following: cardiac, hepatic, renal, osseous, whole blood and blood serum. In addition, certain physical and biological parameters were recorded for each animal. The results were analyzed as a 2 x 2 factorial, segregating the effects of age and brisket disease, and the age-disease interaction.

The following statistically significant ($P<0.05$) differences were attributed to the effect of brisket disease: reduction in the percent dry matter and percent ash in all soft tissues studied; increase in cardiac, hepatic and renal calcium and sodium; decrease in serum total calcium; marked decrease in hepatic copper and increase in hepatic iron; decreased blood iron, hematocrit and hemoglobin; decreased hepatic potassium, magnesium and phosphorus; and increased hepatic zinc.

The effects of brisket disease are superimposed upon these marked differences in the cattle in the present study as compared to those in a previous study of well nourished cattle of similar breeding from a similar environment: reduced cardiac, hepatic, serum and osseous calcium; reduced hepatic, osseous and serum magnesium and increased renal magnesium; reduced hepatic phoshporus and increased renal phosphorus; reduced hepatic, serum and osseous potassium and increased cardiac potassium; and reduced cardiac, osseous and serum sodium and zinc.

The effects of age must be evaluated in view of the fact that half of the animals were diseased; moreover, some age effects occurred almost exclusively in the diseased animals. Statistically significant ($P<0.05$) differences attributed to the effect of age were: decreased phosphorus concentrations in hepatic and renal tissue and serum; increased percent dry matter in hepatic and osseous tissue; increased osseous percent ash; decreased hepatic and osseous potassium; increased

serum ionic calcium; and decreased hepatic calcium, magnesium and sodium, all in cows as compared to calves.

The interaction of increased age and brisket disease produced the following stastically significant ($P<0.05$) results: hepatic percent dry matter and iron concentration were increased; hepatic magnesium, potassium and sodium were decreased; and cardiac zinc was increased.

Hypotheses regarding possible reasons for these results are formulated and discussed.

(201 pages)

INTRODUCTION

Brisket disease is a cardiopulmonary disease of cattle. It is of interest and importance because of economic losses sustained by the cattle industry, and has special importance to researchers because it provides a model for cardiopathologic research. Each year it affects up to 5% (10) of cattle grazing in endemic areas, at high altitudes in the Rocky mountains of the western United States and the South American Andes.

In Utah, the mortality approaches 100% if afflicted cattle are not transported to a lower altitude, which is difficult and generally impractical because of the primitive nature of the areas in Utah where the disease occurs. The animal may die in the process of moving it, and even if remission is achieved through convalescence at lower altitudes, the animal remains a cardiac invalid for months. There are no practical preventive measures, given the conditions of occurrence of the disease in Utah. Even symptomatic treatment is ineffective as long as an afflicted animal is kept at high altitude (14). Moving all cattle from high altitude ranges would solve the problem of occurrence but

would create a greater problem of inefficient use of public grazing lands.

The external signs of brisket disease are lethargy, roughened coat, diarrhea, labored breathing, eroded muzzle epithelium, and edema (14), most notably edema of the brisket, which gives the disease its name. The most striking lesions are ascites and enlargement of the right ventricle of the heart. It is enlarged both in lumen size and in myocardial mass, while the left ventricle is generally little affected (11).

The disease in Utah usually occurs only when cattle remain for some time above 7,000 feet elevation. The practice is to drive or transport cattle in early summer (May or June) from the valleys to the high mountain ranges where they remain until fall. Brisket disease begins to appear in August and reaches peak incidence in September and October.

The symptoms are partially alleviated by transporting a diseased animal to a lower altitude. Therefore, it is apparent that high altitude is one causative factor of the disease. However, there are certain cattle ranges, even as high as 10,500 feet elevation, in Utah where the disease has never been known ot occur (10). The morbidity is not highly correlated with altitude, providing that the basic requirement of at least 7,000 feet elevation is met. There is evidently a causative factor or factors in addition to altitude to account for the inconsistent epidemiology.

The highest incidence of brisket disease in Utah occurs where there are many subalpine marshy meadows, while the lowest incidence (usually approaching zero) occurs on browse type range with rough terrain (10). The dominant forage species, mostly sedges and rushes, in the marshy meadows are nutritionally deficient in calcium and sodium contents and excessive in potassium content for cattle. Oxalate content of several of the species is high (1). It has been demonstrated that cattle with brisket disease have lower blood calcium levels (hypocalcemia) and higher blood potassium levels (hyperkalemia) than healthy animals from the same herd (12). Thus, Blake has postulated (13) the dual-stress theory of brisket disease, which imputes a combination of altitude-induced hypoxia and an imbalance of ions which regulate the contractile efficiency of the myocardium.

An attempt by Bailey and Blake, at Utah State University, to induce brisket disease in healthy cattle by imposing the dual stresses of high altitude and induced ion imbalance (hypocalcemia and hyperkalemia) was met with partial success and was followed by tissue chemistry studies on the experimental animals (7). The study provides a control group for normal blood and organ mineral levels of Hereford calves at 9,000 feet elevation and provides data on the effect of altitude increase on the mineral composition of various tissues of healthy cattle.

The present research is an investigation of the mineral contents of tissues of cattle with naturally-occurring cases

of brisket disease, compared to healthy cattle from the same herds. Because brisket disease frequently develops semi-acutely in calves, but nearly always develops chronically in mature cattle, the experimental animals have been chosen to provide four groups, as follows: 1) calves and 2) cows with brisket disease, and 3) healthy calves and 4) healthy cows from the same herds. In order to minimize the differences of heredity and environment, we obtained when possible the healthy dam of each brisket calf and a healthy calf from each brisket cow. The results were analyzed as a 2 x 2 factorial, segregating the effects of age and disease, and the age-disease interaction.

It is hoped that the results will contribute to brisket disease research by either supporting or challenging the dual stress theory of the cause of brisket disease, and by revealing useful information relative to implementation of better control methods.

REVIEW OF LITERATURE

Brisket Disease: Description, and Theories of Cause, Occurrence, and Control

The pioneer publishing investigator of brisket disease was G. H. Glover of the Colorado Agricultural Experiment Station. He reported in 1913 (32) that the disease had been known in the high mountain ranges for several years, was evidently becoming more common, could not be transmitted by inoculation, and often could be alleviated by removing the animal to lower altitudes but not by medicinal treatment. In 1914 (33) he reported the principal physical symptoms: listlessness, diarrhea, rapid weak pulse, no fever, edema in prolonged cases, and pronounced jugular pulsation following exercise. He reported the disease to be less easily recognized in calves because they swell less.

At necropsy he described the principal signs of brisket disease: enlarged heart, fibrous liver, gelatinous edema deposits and a large quantity of ascitic fluid. He knew that altitude is the predominant cause but discounted the effect of diet. He suggested the use of breeding to reduce the incidence, which is still practiced.

Since then, the disease in cattle has been reported in Utah (10), Wyoming and New Mexico (34), Idaho and Montana (15), and California (45) in he U.S.A., and in Colombia (75), Peru (25) and throughout the South American Andes. It has also been reported in sheep (23, 24, 56, 75).

Since Glover's time, many papers have been written describing the symptoms of brisket disease and often postulating a cause, but no one has been able to support a theory of etiology with convincing proof. Blake (13) described an allergen theory, which suggests that the pulmonary hypertension which causes the right heart failure is a result of a temporarily increased resistance to pulmonary blood flow brought on by exposure to certain toxins or allergens in endemic areas. This would explain the seasonal incidence of the disease and the fact that it occurs most frequently in wet meadowlands where certain plants flourish. The allergen theory has never been seriously investigated. The same circumstances of seasonal occurrence and higher incidence in certain herds grazing specific areas, suggest that the disease may be infectious, but attempts to isolate a pathogen or transmit the disease from animal to animal, beginning with Glover's attempts in 1913, have all proven fruitless. Hecht et. al. (40) believed the disease to be the direct result of a pulmonary hypertensive response to anoxia, due to an extraordinarily thick muscularis encircling bovine pulmonary arteries, even those as small as 20 microns in diameter. They offer this anatomical peculiarity as the

explanation for the fact that mountain sickness in cattle is manifested differently from mountain sickness in man. Pierson (59) believed that pneumonia or some other pathological condition of the lungs, occurring with greater frequency in the cold autumn months, would incite the disease.

Maunder (55) described a syndrome similar to brisket disease occurring in cattle in Australia (St. George Disease) and drew parallels between the symptoms and those of cobalt deficiency. He made no mention of altitude and asserted that treatment with cobalt, copper and iron was successful. This disease is now generally recognized as a separate entity from brisket disease. One researcher (19) associated brisket disease with iron and copper deficiency and the presence of vanadium. Blake (13) argued convincingly for the theory that the combination of high altitude, hypocalcemia and hyperkalemia in Utah cattle afflicted with brisket disease overwhelms the heart. Abaza (1) gave support to this theory with evidence that the plant species in the areas of Utah where brisket disease is endemic are deficient in calcium and sodium, excessive in potassium, iron, manganese and copper, high in oxalate, which would tend to reduce available calcium, and so lacking in cobalt and molybdenum that quantities of these elements were not measureable.

The fact that there are heavier losses during wet years (50) could support almost any of the causative theories. A hormonal alteration due to castration could be a factor, as steer calves are much more susceptible to brisket disease

than are heifer calves, while there is apparently no sex difference in incidence of the disease in cows and bulls (10).

There is dispute as to whether animals which have recovered from the disease are protected from a recurrence. Madsen (50) asserted that affected calves never survive another year on the range. Blake (11) reported that of 20 cattle which developed brisket disease, convalesced for a year at low altitude, and then returned to the same high altitude ranges where the disease occurred, only one redeveloped the disease.

Treatments have included mineral supplements, lucerne hay (61), vitamins (81), diuretics, digitalis, electrolyte therapy, antihypertensive drugs and tranquilizers (14). Intensive treatment such as a human cardiac patient would receive is admittedly effective, but not a practical or economic therapeutic regimen for brisket disease.

Brisket Disease and Mineral Metabolism

The hypothesis that high altitude and toxic dietary mineral imbalances combine to induce brisket disease (13) is compelling and readily lends itself to investigation. Bailey (7) studied the influence of high altitude and experimentally induced mineral imbalance in Hereford calves. Although the experimental conditions failed to induce classical brisket disease, the results provide excellent control data for the expected levels of minerals in the tissues,

determined by the same methods used in the present project. The effect of increased altitude on many of the parameters in the present study is reported by Bailey. His project also includes a well-nourished control group at 9,000 feet elevation, which is close to the altitude at which many of our experimental animals were pastured. Further information regarding his project is provided on page 66 and in Table 5.

The following is a discussion of the physiological roles and the effects of excesses and deficiencies of nutritional and hematinic elements. Normal tissue levels and the effects of increased altitude and increased age are reported when known.

Calcium

Calcium is the most abundant mineral element in the mammalian body. About 99% of the total body calcium is in the skeleton (77). The other 1% is distributed in intracellular and extracellular fluids and plays essential roles in nerve and muscle functioning and blood coagulation.

A homeostatic level of calcium is normally maintained in the body fluids by parathyroid hormone, which causes increased calcium absorption from the gastrointestinal tract and decreased renal tubular resorption of phosphate; and calcitonin from the thyroid gland and ultimobranchial tissue, which prevents hypercalcemia by increasing the deposition of calcium in bone (20). Since the product of calcium concentration times phosphate concentration in body fluids is a

constant, a parathormone-induced decrease in blood phosphate concentration is followed by an increase in blood calcium which is dissolved from the bones. Similarly, an increased phosphate concentration in the vicinity of growing bone would produce an excessive local phosphate product, resulting in calcium phosphate precipitation. Recent evidence (59) indicates that "seed crystals" of hydroxyapatite are produced within the osteocyte itself and either transferred to the bone matrix when extracellular calcium and phosphate are plentiful, or released into the extracellular fluids when necessary. This is a considerable advancement over the previous theories, which characterized the calcium-phosphate solubility product itself as the ultimate determinant of calcium deposition or redissolution.

Calcium acts as a stabilizing factor in nerves and muscles. Extensive literature shows that decreased calcium causes increased membrane permeability in all types of cells (51). In most cases the inverse is also true: excess calcium reduces responsiveness. Hypercalcemia can cause coma; hypocalcemia can cause tremors and convulsions (21).

The action potential of a nerve is transmitted along the axon as an influx of sodium followed by an efflux of potassium, creating an electrical current. A common representation is that calcium regulates neuronal permeability to sodium and potassium by a mechanical means, being bound to the axonal membrane at rest and effluxing at the beginning of the action potential. However, the quantities of calcium

involved are so small that definitive studies concerning the exact mechanism of calcium's influence on axon permeability have yet to be performed (51).

The action of calcium in muscles is better delineated. During rest, calcium is bound to the sarcolemma, where, as in nerves, it regulates the sodium-potassium permeability, and thus the excitability. At the beginning of contraction, it is released from the sarcolemma and sarcoplasmic reticulum (which is highly developed in skeletal but minimal in cardiac muscle) into the sarcoplasm (17), where it acts via the troponin-tropomyosin complex (78) to initiate the contractile process. The intensity of the contraction is directly proportional to the intracellular calcium at this time. After contraction, calcium is again bound to the sarcoplasmic reticulum, which is called the "relaxing factor" because removal of calcium permits the muscle to relax. The rapidity of the rebinding process determines the duration of contraction (17). Thus all motion in the body is utterly dependent upon calcium concentration. It is not surprising that there are two hormones to control its levels and vast stores in the bone to supply it in times of deficiency.

According to Thomas and Howard (69), calcium deficiency can occur in diets lacking in calcium, or vitamin D, which is necessary for its absorption, or excessive in fats, which tend to form insoluble calcium soaps. Dietary oxalate can also cause calcium deficiency (1). Nondietary causes are parathyroid deficiency, malfunction of the calcium

resorption mechanism of the renal tubules, acidosis, and hyperadrenocorticism. In many cases, blood levels are kept normal by homeostatic mechanisms and hypocalcemia does not occur, but rickets or osteoporosis may develop. When calcium deprivation is sudden, as in oxalate poisoning or infusion of another chelating agent such as citrate or ethylenediaminetetraacetic acid (EDTA), or surgical removal of the parathyroids without replacement therapy, severe hypocalcemia develops before bone calcium stores can be mobilized, and convulsions and heart failure may occur. Tremors and convulsions may have a therapeutic effect, as they cause large quantities of lactic acid to be produced by the muscles, and acidosis helps to mobilize bone stores of calcium.

Thomas and Howard (68) indicate that hypercalcemia due to dietary excess is almost never encountered. Hyperparathyroidism, hypervitaminosis D, and the ingestion of large amounts of absorbable alkali in conjunction with large amounts of calcium ("milk alkali syndrome") can cause hypercalcemia. The symptoms are nausea, vomiting, polyuria, polydipsia, pruritis, and often metastatic calcification.

Bailey (7) found calcium levels in well nourished Hereford calves at 9,000 feet elevation to average 9.5 mg ionic calcium per 100 ml of blood serum, and recorded these tissue levles of calcium: 235.0 parts per million (ppm) in cardiac, 286.9 ppm in hepatic, 286,5 ppm in renal tissue, all on an absolute dry matter basis; and 32.47 percent of ash in bone. Blake (12) found serum ionic calcium levels of 5.52 mg/100

ml in cattle severely afflicted, and 7.70 mg/100 ml in cattle moderately afflicted with naturally occurring cases of brisket disease, and 8.53 mg/100 ml in healthy cattle from the same herds. One would expect, then, that the severely diseased animals had severe chronic dietary calcium deficiency, or deranged hormonal control mechanisms, for their concentrations to fall so low as to border on convulsive levels. Such severe hypocalcemia should lead to reduced myocardial contractility and heart failure, the cardinal manifestation of brisket disease.

According to Bailey (7) (see also Table 5), calcium levels in serum, hepatic and osseous tissues in well nourished Hereford calves after four months at 9,000 feet elevation are not significantly different from those in cattle fed the same diet and kept at 4,500 feet. Cardiac calcium concentration is significantly lower and renal calcium is significantly higher in the high altitude group. The 95% confidence level is taken as the minimum criterion for significance throughout this dissertation.

Serum calcium in the bovine appears to remain constant or decrease slightly with increasing age (4, 47). Hepatic calcium is twice as concentrated in the neonate as in the adult, and the calcium concentration of skeletal muscle also decreases with increased age (77). The proportion of the total body calcium which is in soft tissues decreases with increasing age, as the skeleton increases in mass (77).

Chloride

Chloride is seldom considered a nutritionally or toxicologically important entity, since most diets contain ample quantities of it, and dietary chloride excesses cause no known health problem. However, chloride is the major anion in extracellular fluids. It maintains the acidity of gastric secretions and plays a vital role in carbon dioxide transport by the red blood cell. After carbon dioxide is converted to bicarbonate ion by carbonic anhydrase within the red blood cell, the bicarbonate diffuses out of the cell and chloride diffuses in. This is known as the chloride shift. Thus, red blood cells in venous blood have a higher chloride content than arterial red blood cells. In the lungs, chloride diffuses out of the erythrocyte and bicarbonate diffuses in and is reconverted to carbon dioxide, which passes into the alveoli and is excreted (22).

According to Cotlove and Hogben (22), body chloride is depleted by diabetic acidosis, adrenal cortical insufficiency, and excessive vomiting, and increased in congestive heart failure. The other metabolic derangements occurring with these syndromes are considered more important than changes in chloride in producing pathology. Thus we have very little idea of what the simple effect of chloride deprivation or chloride excess would be.

Bailey (7) found the serum chloride concentration of the well nourished five to seven month old Hereford at 9,000 feet elevation to average 397.0 mg/100 ml. This was an

increase over the serum chloride levels in well nourished calves at 4,500 feet, but the difference was not statistically significant at P=0.05. Serum chloride increases slightly with age in the heifer (4).

Cobalt

Cobalt is vital to organisms because it is an integral part of the structure of vitamin B 12, cyancobalmin, which is necessary as a cofactor in basic enzyme reactions such as transmethylation. Smith (66) asserts that some organisms have astonishingly low requirements for cobalt. Simple stomached animals do not need nearly as much dietary cobalt as ruminants, which depend on rumen microorganisms to produce B 12 from cobalt (66). These microorganisms utilize a high percentage of dietary cobalt to synthesize other compounds of no value to the ruminant. The recommended dietary level of cobalt for the ruminant is 0.1 to 0.3 parts per million parts of dry forage (48).

Dietary cobalt deficiency, seldom manifested except in the ruminant, is indicated by loss of appetite, poor body condition, listlessness, watery eyes and roughened coat. Severe anemia and the appearance of starvation may ensue (31). The condition is known in different areas of the world as pine or pining sickness, daising, vinquish, coast disease, Burton ail, Denmark disease, bush or salt sickness, enzootic marasmus, or nakuritis (66). Some of these are the result of deficiencies of other elements as well as cobalt

(66). Even borderline cobalt deficiency will reduce growth and weight gain (31).

In the United States, cobalt deficiency in forage is almost universal in Florida, New Hampshire, and Michigan, and has been reported in 15 other states (31). Cobalt deficiency syndrome has not been reported in Utah, according to Fuller and McAlpine (31), but Abaza's work (1) indicates that the forage in endemic areas of brisket disease may be cobalt deficient.

Cobalt deficiency in the ruminant can be cured by feeding cobalt so the rumen microorganisms can produce vitamin B 12, or by injecting vitamin B 12. Either method is far more effective than injecting cobalt salts; therefore the signs observed in cobalt deficiency should be ascribed to deficiency of vitamin B 12 rather than simple cobalt deficiency (66).

Administration of excess cobalt salts can induce polycythemia in a number of species. The apparent mechanism is production of a state of anoxia, followed by homeostatic erythropoiesis (66). The suggestion that cobalt should be used as a symptomatic treatment for anemia has not been enthusiastically received by the medical community.

Dietary or medicinal excesses of cobalt can produce polycythemia, weight loss, listlessness, roughened coat and loss of appetite. Large quantities are required to produce these symptoms in ruminants (66). Recent reports (37, 48, 67) indicate that excessive cobalt produces cardiomyopathy,

with myofibrillar degeneration, interstitial edema, cellular infiltration and finally widespread ventricular myofibrillar damage. But according to Abaza (1) we would not expect to find excessive cobalt levels in any of our animals.

Bailey (7) did not give values for cobalt in his animals. He reportedly (8) analyzed for cobalt, but with indeterminate results. According to Rothery (64), the liver is the main storage organ for cobalt. He found 100 to 200 micrograms of cobalt per kilogram of liver, and one microgram per kilogram of whole blood in lambs fed 500 micrograms of cobalt per day. There have been no reports in recent literature concerning changes in tissue cobalt levels with altitude.

Cobalt concentrations in human serum do not change with increasing age in the adult human being (18). The present study is, ostensibly, the first to consider the effect of age on bovine cobalt levels.

Copper

Copper is a constituent of, or essential for the functioning of, the following enzymes: cytochrome oxidase, catalase, tyrosinase, monoamine oxidase, ascorbic acid oxidase and uricase (2). It is essential for the utilization of iron in the production of hemoglobin, and promotes the absorption of iron from the gastrointestinal tract. Human red blood cells contain 30 to 36 mg/100 ml of colorless erythrocuprein, serum contains 30 mg/100 ml of blue ceruloplasmin, cerebrocuprein is found in the brain (39), and hepatocuprein in the liver (2). The function of these four copper-containing

proteins is a matter of controversy, but ceruloplasmin is known to be an oxidizing enzyme.

Calcium prevents and hydrochloric acid promotes the absorption of copper (2). According to Gubler (38), copper homeostasis is accomplished by adjusting the rate of excretion to match the variable rate of absorption of copper.

The liver is the main storage site for copper, and hepatic copper levels in cattle and sheep are a direct reflection of dietary copper intake (2). Excess copper leads rapidly to toxicity, and dietary deficiency to depletion of liver copper stores in the ruminant. The recommended dietary copper level for beef cattle is 2 to 4 mg of copper per pound of air dry ration, or less if the ration is low in molybdenum and sulfate (58).

Adelstein and Vallee (2) describe a number of interactions between copper and other cations. Excess molybdenum intake leads to a bovine disease called "teart", with all the symptoms of copper deficiency, which can be cured either by increasing dietary copper or decreasing molybdenum. Low dietary molybdenum in sheep leads to copper intoxication, while excess molybdenum can induce copper deficiency. The anticupric toxicity of molybdenum is enhanced by ample dietary sulfate. Some report (2) that it is reduced by excess manganese. Apparently the mode of action of molybdenum involves complexation with copper and an interference with copper uptake by hepatic cells, where ceruloplasmin is synthesized (5). Blood copper levels are high and large

quantities of copper are excreted in the presence of excess molybdenum (2).

There are also interactions between copper and zinc: excess dietary zinc reduces liver cytochrome and catalase activities and produces anemia, stunted growth and reproductive failure (2). Supplementary copper restores enzyme activity and, with iron and cobalt, can reverse the anemia. Excess copper can decimate liver zinc stores (2).

Without adequate copper, iron cannot be incorporated into hemoglobin. The resultant anemia is hypochromic in cattle (2). Cattle and sheep grazing copper deficient forage accumulate extensive hepatic iron deposits (52). Recent reports indicate that the interrelationship is dependent upon the ferroxidase activity of ceruloplasmin, which catalyzes the oxidation of Fe(II) to Fe(III) for incorporation into transferrin, the form in which iron is available to the tissues and erythropoietic bone marrow (30).

The effects of copper deficiency, whether it is simple, or secondary to imbalances with other metals, are, generally, hypochromic anemia, osteoporosis, neonatal ataxia in the offspring of copper-deficient dams (due to prenatal demyelination or failure to myelinate), various abnormalities in pigmentation and hair quality, and, in cattle, myocardial fibrosis, diarrhea, and scours (2). The bovine myocardial fibrosis, which occurs mainly in Australia, results seasonally in a sudden death syndrome known as "falling disease". Cardiac lesions include cellular infiltration and large, widely

distributed areas of atrophy of the myocardium, leading to displacement by collagen (2). Aneurism and hemorrhage have also been observed in copper deficient swine and sheep, and cardiac hypertrophy in copper deficient pigs (2).

Copper toxicity is rare in cattle. Whether a toxic excess is acute or chronic, the result tends to be accumulation of high concentrations of copper in the liver and other tissues, followed by sudden liberation of the hepatic copper stores, resulting in an acute hemolytic episode which can be fatal (71).

Bailey (7) found copper levels of 22.38 ppm in cardiac and 82.86 ppm in hepatic tissue, dry matter bases; and 107.5 micrograms (ug) per 100 ml in blood serum of his well nourished Hereford calves at 9,000 feet elevation. There were no significant altitude-induced changes in copper concentrations in Bailey's animals.

The liver copper concentration in the human newborn is more than ten times as great as that in the one year old (2). After one year, there is little subsequent change. In the kidney, spleen, and heart there is also some decrease in copper concentration during infancy. The diminution in copper levels in soft tissues occurs in all common animals except the sheep (2). When soft tissue copper levels decrease in infancy, serum copper concentration increases (2).

Iron

Moore and Dubach (57) give the following basic information concerning iron metabolism: it is a constituent of

hemoglobin, the primary oxygen transport protein; of myoglobin, which scavenges and stores oxygen for the heart and skeletal muscles; and of the catalases, cytochromes and peroxidases. In the dog, 60 to 70% of the body iron is found in hemoglobin and myoglobin, about 20% in labile storage in the liver, spleen and other tissues, and the remaining 10 to 20% is firmly fixed in the tissues. In addition to the above mentioned compounds, which all have the familiar heme structure, iron is also found in ferroflavoproteins such as xanthine oxidase, succinic dehydrogenase, and DPNH-cytochrome reductase, in actin and myosin, in the transport form, "transferrin", the storage form, "ferritin", and the precipitate "hemosiderin".

Perhaps because of the fact that animals have very little capacity to excrete iron, the intestinal absorption mechanism is elaborate. Absorption can occur in the stomach and all through the intestine, but is greatest in the duodenum. Iron is absorbed in the ferrous form, oxidized to the ferric form in the intestinal mucosal cell, and complexed with the protein apoferritin to form ferritin. At the capillary surface of the cell, apoferritin releases ferric iron, which is reduced to ferrous iron and released into the plasma, where it must be reoxidized to ferric iron before it is incorporated into transferrin (57).

While the exact mechanism of control over iron absorption remains in doubt, it is clear that the rate of iron absorption is increased in iron deficiency and when

erythropoiesis is depressed or iron stores are large. Some investigators feel that the rate-controlling factor is the amount of unbound transferrin in the plasma, but there is convincing evidence against this hypothesis (57).

Adequate iron is provided to male Holstein calves if the diet contains 30 mg of iron per day (54). Dietary iron deficiency is almost unknown in ruminants, except in calves on an exclusively milk diet, in which the resultant anemia is easily cured by supplemental iron. A moderate dietary excess of iron causes little problem because the iron is not absorbed in toxic quantities. An extreme dietary excess of iron results in precipitation of iron pigments in the liver, leading to hepatic fibrosis in some cases (57).

Bailey (7) found the following iron concentrations in his well nourished Hereford calves at 9,000 feet elevation: 252.5 ppm in liver, 212.8 ppm in cardiac tissue (both on a dry matter basis), and 53.0 mg/100 ml in whole blood. Cardiac and hepatic iron concentrations were increased insignificantly, while hemal iron levels were increased significantly with increased altitude in Bailey's study.

Total body iron changes with age, but the direction of change varies according to species (71). There is no specific information on the direction of the change in the bovine, except Greatorex (36) reported that blood iron levels generally decrease in calves between birth and one year of age.

Magnesium

Thorén's monograph on magnesium (70) provides the

following information: over one-half of the magnesium in the body is found in bone. The rest is primarily intracellular: for example, red blood cells contain three times as much magnesium as does serum. Magnesium is a cofactor to many enzymes, including the phosphatases, hexokinase, cholinesterase, cocarboxylase, transphosphorylase, and enolase. It is essential for DNA replication and mitosis, and for the integrity and function of mitochondria. In the absence of magnesium, there is an uncoupling of oxidative phosphorylation.

Experimentally administered excessive magnesium causes central nervous system depression and local anaesthesia sufficient to perform major surgery (76). Magnesium reduces neuromuscular transmission in the peripheral nervous system (16). Hypomagnesemia can produce tetany. Although hypocalcemia also produces tetany, which suggests that calcium and magnesium perform a similar function, paralysis due to hypermagnesemia can be immediately relieved by the administration of calcium (35). Magnesium is thought to interfere with, and calcium to be essential for, the release of acetylcholine from motor nerve terminals (35). Excess magnesium causes reduced myocardial contractility and electrical conductivity, and a resultant reduction in blood pressure (76).

The absorption of magnesium from the gastrointestinal tract is described by Thorén (70). Absorption is reduced in the presence of excess calcium and increased in the absence of calcium, indicating a competitive absorption pathway for

the two cations. The proportion of ingested magnesium which is absorbed is increased when the total dietary intake is low, and decreased when the total dietary intake is high; however, no specific absorption controlling factor or mechanism has been identified. Renal excretion of magnesium increases dramatically after parenteral administration of magnesium. The excretion is also increased in primary hypoaldosteronism, to the extent that serious depletion of body stores of magnesium may occur (76).

Magnesium deficiency is common in cattle. It can occur in calves receiving only milk and in cattle grazing magnesium deficient forage (76). The latter is known as grass tetany and is often associated with excess potassium as well as deficient magnesium in the forage (68). The symptoms are failure to grow, skin lesions, neuromuscular hyperirritability, exhaustion, fasciculations, falling blood pressure, convulsions and death (70, 76). In less acute cases, calcification of the endocardium, the aorta, and other large arteries may occur (76). Experimental magnesium deficiency in rats is accompanied by hypercalcemia, hypophosphatemia, and an increased excretion of phosphate in the urine, all thought to be the result of a compensatory increase in parathyroid activity (6). There is also a loss of potassium from the sarcoplasm, ascribed to a reduction of magnesium-dependent ATPase activity, which is necessary for active transport and thus for the maintenance of the sodium/potassium gradient across the sarcolemma (35).

Naturally occurring dietary magnesium toxicity has not been reported in cattle. However, the reduced dietary calcium availability in areas endemic to brisket disease in Utah (1) could favor increased magnesium absorption.

Bailey (7) found the following magnesium levels in his well-nourished Hereford calves at 9,000 feet elevation: 731.9 ppm in liver, 930.3 ppm in cardiac tissue, and 517.6 ppm in kidney, all on a dry matter basis; 2.54 mg/100 ml in serum, and 0.776 percent (ash basis) in the right metatarsal bone.

Cardiac and hepatic magnesium levels were increased, while serum, renal and osseous levels were decreased, in the high altitude group in Bailey's study. The decrease in bone magnesium was the only statistically significant altitude-dependent change in this element.

During human growth, magnesium concentration does not change in skeletal muscle, but decreases in the liver (77). Plasma magnesium decreases with age in the bull, while changes in the female bovine are small and inconsistent (4).

Molybdenum

Molybdenum is an integral part of xanthine oxidase, aldehyde oxidases and other flavoenzymes (26). While artificial diets severely deficient in molybdenum result in reduced xanthine oxidase levels (62), no gross effect or deficiency syndrome due solely to the lack of molybdenum has been reported.

The copper-molybdenum-sulfate antagonism has been

described under copper (p. 18 in this treatise). Excessive dietary molybdenum leads to the copper deficiency syndrome known as "teart", while inadequate molybdenum levels produce copper toxicity. With stable copper and molybdenum intake, increased sulfate reduces tissue molybdenum levels (71).

Molybdenum also has an antagonistic relationship with tungsten, described by DeRenzo (26). Tungsten is a competitive inhibitor to the utilization of molybdenum in the formation of xanthine oxidase. Tungsten surfeit, like simple molybdenum deficiency, results in reduced xanthine oxidase levels but no overt pathology, except in the chick, which relies on xanthine oxidase to metabolize nitrogenous substances to uric acid for excretion. It seems likely that other organisms can resort to alternate metabolic pathways when xanthine oxidase is depleted.

Experimentally induced dietary molybdenosis in rabbits produced weight loss, anemia, epiphyseal fragility and, in some cases, degeneration of cardiac and skeletal muscle (74).

Molybdenum increases the absorption and excretion of phosphorus and decreases liver copper stores. It is rapidly absorbed and rapidly excreted in the urine. Sulfate is necessary for the renal excretion of molybdenum (26).

While these effects and interactions may have significance in the present study (see discussion section), no specific relationship of molybdenum to brisket disease can be proposed at the present time.

Underwood (71) reports whole blood molybdenum

concentrations of 1 to 6 ug/100 ml in sheep and cattle grazing pastures normal in copper and low in molybdenum contents. Increasing the dietary intake to 30 ppm molybdenum increased blood molybdenum levels to 60 to 80 ug/100 ml in young cattle.

Molybdenum levels in human whole blood varied widely, depending upon the residence of the subject, from less than 0.5 ug/100 ml to 41.0 ug/100 ml in a recent survey (3).

Liver molybdenum levels are highly susceptible to dietary influence, ranging from 2 to 4 ppm on a dry matter basis for several species under average dietary conditions, to 25 to 30 ppm on a dry matter basis in sheep and cows ingesting moderately large amounts of molybdenum (71). The liver molybdenum levels rapidly returned to normal when the administration of excess molybdenum ceased (71).

No consistent effects of age or altitude on blood or liver molybdenum levels have been reported.

Phosphorus

Phosphate is the principal anion found in bone and the most abundant inorganic ion in cells. Phosphate compounds are the medium of exchange of energy in living systems. Nucleic acids are joined by phosphate bonds. Phospholipids are a basic structural component of biological membranes. Phosphorus is essential for carbohydrate and fat metabolism, renal mediation of acid-base balance, and, ultimately, all energy-requiring metabolic processes (33).

Phosphate metabolism is generally related to calcium metabolism. The product of calcium concentration times phosphorus concentration in the extracellular fluids tends to be constant. An excess of either increases the elimination of the other via parathormone and thyrocalcitonin mediated control mechanisms (43).

Adequate phosphorus is provided to growing beef cattle if the ration contains 0.25% phosphorus on a dry matter basis. Dietary deficiency leads to malcalcification and eventually to frank rickets (43). Soft tissue phosphate levels are maintained at the expense of bone, and nonosseous effects of phosphate deficiency are minimal.

Excess dietary phosphate is absorbed and rapidly excreted. Large excesses escape absorption and have a cathartic effect. Parenterally administered excess phosphate produces calcium deficiency (35).

Bailey (7) found the following phosphorus levels in his healthy Hereford calves at 9,000 feet elevation: 16.14 parts per thousand (ppt) in liver, 8.42 ppt in heart, and 7.55 ppt in kidney, all on a dry matter basis; 5.28 mg/100 ml in serum and 19.38 percent of ash in right metatarsal bone. In the same group, phosphorus levels were increased over those in the 4,500 feet elevation control group in every tissue studied: serum, heart liver, kidney and bone. The renal and osseous increases were statistically significant.

Phosphorus concentrations in the skeletal muscle and hepatic tissue of men decrease with age (77). The

percentage of the body phosphorus in the soft tissues decreases as the skeleton grows (77). Plasma phosphorus in the bull, cow and heifer decreases with increasing age (4, 47).

Potassium

The following information about potassium is elaborated upon in Wilde's review (79). Potassium is the chief intracellular cation. Active transport processes at the cell membrane continuously accumulate potassium and eliminate sodium from the cell. The temporary breakdown of this barrier provides the electrical basis for neural conductivity and muscular contraction. Changes in extracellular potassium concentration can profoundly affect conductivity and contractility: decreased extracellular potassium acts as a membrane stabilizer, and muscular weakness ensues; whereas excess potassium causes the heart to become extremely dilated and flaccid and slows its rate. Because animals with brisket disease are known to have hyperkalemia, this has been incriminated as a possible causative factor.

Carbohydrate metabolism is an important determinant of extracellular potassium levels: during glycogenolysis the liver liberates potassium as well as glucose. Glycogen synthesis requires hepatic accumulation of potassium. Amino acid uptake by growing tissue also requires the incorporation of potassium, and catabolism releases potassium (79).

The kidney is capable of efficiently excreting large quantities of potassium by active secretion as well as by

simple failure to resorb it from the glomerular filtrate. Aldosterone and other mineralocorticoids increase renal potassium clearance. Nevertheless, ingestion of massive quantities of potassium could cause hyperkalemia, with cardiac and central nervous system depression, bradycardia, flaccid paralysis and death. In contrast, hypokalemia is characterized by muscular weakness, irritability, and paralysis; and by tachycardia with cardiac distension and similar risk of death (79).

Bailey (7) found the following potassium levels in his healthy calves at 9,000 feet altitude: 14.49 ppt in liver, 5.230 ppt in heart, 10.28 ppt in kidney, all on a dry matter basis; 7.8 meq/l in blood serum, 14.4 meq/l in whole blood, and 0.0872 percent of ash in right metatarsal bone. Insignificant decreases in blood, liver and kidney potassium, and a highly significant loss of osseous potassium, were contrasted by a significant increase in serum potassium and no change in cardiac potassium in the 9,000 feet altitude groups, compared to the 4,500 feet altitude groups in Bailey's study.

Serum and whole blood potassium in the neonatal heifer decrease steadily during the first three months of life (4). In the bull, serum potassium increases slightly from 2 to 15 years of age (4), while whole blood potassium increases irregularly but significantly (47). From birth to adulthood the potassium concentration of human sleletal muscle is halved, while that of liver is doubled (77).

Sodium

Sodium is the most abundant cation in the extracellular fluid. With potassium, it is essential to the electrical function of nerve and muscle. Increased extracellular sodium diminishes the strength of myocardial contraction, and decreased sodium increases contractile strength. Maintenance of proper pH and of body fluid volume is largely dependent upon the presence of sodium. Although potassium concentration exceeds sodium within the cell, nuclei and mitochondria maintain a sodium-dominant environment which is optimal for the activity of certain enzymes. Other enzymes are inhibited by sodium in concentrations approaching those in extracellular fluids (29).

Sodium is absorbed from the stomach and intestines and even from the intact skin, according to Forbes (29), from whom the following information is derived: the principal organ of excretion is the kidney, although large amounts can be lost in the sweat and small amounts are routinely excreted in the stool. Aldosterone and mineralocorticoids promote renal sodium retention, while vasopressin, oxytocin and antidiuretic hormone increase urine concentration. However, the ability to concentrate sodium in the urine is not unlimited. The fact that the sea is too salty to be a satisfactory source of drinking water is a result of this limitation.

A completely sodium-free artificial diet results in failure to grow, softening of the bones, corneal keratinization, gonadal inactivity, and, eventually, death, even

though sodium excretion is reduced to meager levels within two to four days. Acute hyponatremia causes decreased cardiac output, decreased mean arterial blood pressure, increased circulation time, increased arterio-venous oxygen difference, and increased hematocrit (29).

Immoderate sodium intake becomes serious when it exceeds the capacity of hormone-augmented renal excretion to purge it. This occurs readily if the renal or endocrine systems are impaired. The result of sodium overload is edema, increased strain on the heart, intracellular dehydration, and potassium loss (29). Drinking exclusively seawater is known to be rapidly fatal.

Bailey (7) reported the following sodium levels in his well nourished Hereford calves at 9,000 feet altitude: 4.59 ppt in liver, 3.48 ppt in heart, and 10.60 ppt in kidney, all on a dry matter basis; 156.3 meq/l in blood serum, 129.3 meq/l in whole blood, and 1.028 percent of ash in the right metatarsal bone. He found insignificant increases in blood and serum sodium and a significant increase in renal sodium, with significant decreases in osseous and hepatic sodium and an insignificant decrease in cardiac sodium, in his high altitude well nourished group compared to his moderate altitude well nourished group.

The sodium concentration in both whole blood and serum of the bovine remains remarkable constant throughout life, in all studies except one, which shows the whole blood sodium of the heifer increasing during the first three months

of life (4). In soft tissues, sodium concentration declines with age in most species, while in bone it increases markedly (29).

Zinc

Zinc is a component of insulin, carboxypeptidase, carbonic anhydrase, glutamic dehydrogenase, lactic dehydrogenase, and alcohol dehydrogenase, and a cofactor to a profusion of other enzymes. It is concentrated in normal leukocytes and conspicuously decreased in leukemic leukocytes. It is highly concentrated in the choroid of the eye, and concentrated in and essential for the function of the male reproductive system (72).

In the well nourished individual the preponderance of dietary zinc is excreted unabsorbed. There is a competition between simultaneously administered copper and zinc for absorption (2), and high dietary calcium levels can reduce the amount of zinc absorbed from a zinc deficient diet (42). Parenterally administered zinc is most frequently excreted through the gastrointestinal tract (72). Urine normally contains a small amount of zinc, which may be increased coincident with albuminuria (72).

Zinc deficiency induces loss of appetite, growth retardation, blood disorders, reproductive failure, and abnormalities of the skin and hair coat. In swine the syndrome is fatal and is known as parakeratosis (73).

Zinc toxicity manifests itself as impaired growth and

anemia which can be fatally severe. A probable mechanism is interference with copper utilization, which results in impaired iron metabolism (72). With acute parenteral administration of zinc in the dog, one sees lassitude, bloody diarrhea, hindlimb paresis and electrocardiographic changes similar to those in potassium intoxication (72).

Bailey (7) found the following zinc levels in his well nourished Hereford calves at 9,000 feet elevation: 230.3 ppm in liver and 116.4 ppm in heart, both on a dry matter basis; 183.3 ug per 100 ml of blood serum and 218.5 ppm (ash basis) in the right metatarsal bone. Bone zinc decreased significantly and serum zinc decreased insignificantly, while cardiac and hepatic zinc increased insignificantly in his high altitude control group compared to his moderate altitude control group.

After birth, the increasing size of the body accounts for the major part of the increment in body zinc (77). From this we can infer that the zinc concentration in the tissues remains essentially constant with increasing age. In the rat, the liver of the newborn contains as much as 2 to 3 times as much zinc as the adult, but adult concentrations are attained within two weeks postpartum (72).

EXPERIMENTAL METHODS

Experimental Design

This experiment was designed as a 2 x 2 factorial to determine the effects of brisket disease and age on the concentrations of certain mineral elements in heart, liver, kidney, bone, whole blood and serum. There were four experimental groups: cows with brisket disease, calves with brisket disease, healthy cows and healthy calves. The differences between cows and calves were considered the result of age, while the differences between healthy animals and brisket-diseased animals were ascribed to disease. The interaction between age and brisket disease interested us because the disease in calves may be chronic or simiacute, whereas in cows the disease is nearly always chronic.

Experimental Animals

Executing the design

In order to reduce variability due to heredity and environment, we planned to study the healthy dam of each brisket-diseased calf chosen, and a healthy calf of each brisket-diseased cow. This plan was followed except in two

instances: B 12 did not have a living healthy calf, so another calf was chosen from the same herd. B 7 is not the offspring of H 7, nor did he come from the same herd or pasture. He was selected later to replace the calf of H 7, which showed only early signs of brisket disease and was not as fully developed a case as was B 7. Except for these instances, all animals with the same code number are dam-calf pairs. Ascending code numbers indicate progressively later dates of occurrence of the disease during the late summer and autumn of 1969 for every animal except B 7. All experimental cattle were of the Hereford breed, except for H 4 and B 4, which were Holstein-Hereford. We collected 12 cow-calf pairs in all, providing six animals for each of the four experimental groups. Brief descriptions of the animals are included in Table 1 A.

Acquisition procedure

We advised ranchers and fieldmen of south-central Utah that we wanted to secure cattle afflicted with brisket disease along with their healthy dams or calves. The experimental subjects were chosen from the cases reported by them. We requested that candidate animals should not be given supplementary feed or allowed to graze any pasture except in the mountainous areas where the disease developed. This request was complied with for all animals except B 12 and H 12, which were both pastured for a month at 4,500 feet after B 12 developed the disease at 8,300 feet elevation.

Potentially suitable pairs, with one member afflicted

and the other member ostensibly healthy, were examined in the field. If both members were acceptable, as judged by visual inspection to evaluate the degree of brisket disease severity in the afflicted member of the pair, they were secured at the site of occurrence of the disease and transported to Logan. Unfortunately, in 1969 there were far fewer than the usual number of cases of brisket disease. As a result, we did not have as much selection as we would have had in other years, nor could we confine our selection to cattle located in the Fishlake area, which is the most endemic area for brisket disease in Utah. B 12 and H 12 came from Cache County.

The experimental animals were not allowed any feed after they were removed from the native habitat. They were allowed water ad libitum. In Logan, they were weighed and clinically examined as follows: heart rate, respiratory rate, and rectal temperature were determined and recorded. Auscultation of the heart and lungs was performed. Signs of brisket disease, including appearance of the hair coat and muzzle epithelium, edema, diarrhea, jugular pulsation and lethargy, were evaluated. Without delay, each animal was slaughtered and necropsied.

Procedure for slaughter and tissue collection

Healthy animals

The healthy cattle were slaughtered at a local packing plant and the diagnosis of health was confirmed. The heart; lungs; liver; kidneys; spleen; thyroid, parathyroid and

adrenal glands; metacarpal and metatarsal bones and left thirteenth rib were obtained. Any urine in the bladder of an animal was aspirated with a chemically clean pipet and saved in a Whirl-Pak[1]. Whole blood was collected in a Whirl-Pak and allowed to clot. Another sample of whole blood was collected in a heparinized Whirl-Pak. A third sample of whole blood, for hematologic studies, was collected in a plastic tube with 0.1 ml of 30% EDTA per 10 ml of blood. A sample of aliment from the ansa spiralis was also collected in a Whirl-Pak. All these samples were transported to our laboratories for further attention.

The necropsy protocol, recorded for each animal, indicated any unusual findings.

Brisket-diseased animals

The animals afflicted with brisket disease were taken to the Veterinary Science building on the U.S.U. campus and electrocuted. Samples of tissue, urine, blood and aliment were collected as for the healthy animals. Edematous fluids from the body cavities and brisket were also sampled. The carcasses were disposed of at a local by-product plant.

The necropsy protocol, recorded for each animal, indicated the evidence of brisket disease and any unusual findings. The severity of disease and degree of swelling were each rated on an arbitrary scale, from 0 to 5. Zero indicated no evidence of swelling or disease, and 5 indicated maximum swelling or fatally severe.

[1]Scientific Products, Evanston, Illinois.

Exceptions

Brisket cow B 9 died in the truck on the way to Logan. Tissue samples were collected within one hour after death and transported to Logan. Brisket calf B 11 also died in the truck. Tissue samples were collected in Logan within 3 hours after death. In both instances, however, blood samples were obtained at the time of death.

Hematologic Studies

Hematocrit, hemoglobin and red cell concentration were determined on EDTA-anticoagulated blood samples from all animals within 6 hours after collection. The samples from B 6 and B 9 were accidentally clotted and frozen, respectively. For statistical analyses, group averages of these parameters were substituted for individual values in these two cases.

Sample Preparation

Clotted blood was set aside for 4 to 8 hours at room temperature, and if necessary for an additional 10 to 16 hours in the refrigerator, to allow the clot to contract. The serum and loose cells were decanted into chemically clean Pyrex bottles and centrifuged for 10 minutes at 2,000 rpm. Serum was transferred with chemically clean pipets to clean Whirl-Paks and was frozen.

Aliment, whole blood, urine and edema fluid were frozen in their Whirl-Pak containers within 3 hours of collection.

All remaining tissues were cleaned of connective tissue using stainless steel instruments, rinsed free of debris with a small amount of double distilled water, and weighed. The right and left atria were removed from the heart, capacities of the right and left ventricular chambers were measured, and the right and left ventricles were partitioned into free walls and septum by the method of Blake (11). Proportionality of these measurements and of lung, liver, spleen, kidney, and the adrenal and thrroid-parathyroid glands in the healthy cattle and contrasting disproportionality in those afflicted with brisket disease (11) were intended to be further criteria of the status of the experimental animals.

In order to know the microbiological status of the cattle, specimens of the liver, spleen and lungs of each animal were routinely cultured. In addition, samples fron the liver, kidney and spleen were cultured on Madden Darby bovine kidney and on either embryonic tracheal cells or bovine embryonic liver cells to determine whether certain virus diseases might be present. Sections of right and left heart ventricle free walls, liver, spleen, left lung, kidney, and thyroid-parathyroid and adrenal glands were taken for possible histologic examination at a later date.

The entire right kidney, the interventricular septum of the heart, a portion of the liver, one half of the thyroid-parathyroid mass and the entire right adrenal gland were sealed in Whirl-Paks for later chemical analyses. Of these, the thyroid, parathyroid and adrenal tissues were frozen

whole within 6 hours after slaughter; the heart, liver, and kidney tissues were minced with stainless steel scissors and processed in a stainless steel Waring Blender to produce uniform homogenates, then frozen in Whirl-Paks. Cardiac tissue tended to resist homogenization and the result was chunks of tissue of 1 cm or less in cross section, interspersed in tissue fluid. The less homogeneous hearts presented some problem in choosing uniform samples for analysis later in the course of these experiments.

The diaphysis of the right metatarsal bone was cut transversely into rings of 3 to 5 mm thickness using a band saw with a carbon steel blade. The two outermost rings containing a marrow cavity, and one ring from approximately the center of the shaft were saved for a bone density determination. The other ring segments were sealed in Whirl-Paks and frozen. The right and left metacarpal bones were split with the band saw and the marrow was removed, using stainless steel and Teflon-coated instruments, and frozen in Whirl-Paks. The left metatarsal bone and right thirteenth rib were frozen intact, as reserves.

After bone density determinations, the three rings used for this, along with the other rings of bone from the right metatarsal, were further cut into segments small enough to fit in the hopper of a stainless steel Wiley mill for grinding at a later time. These segments were next processed for 18 to 24 hours in a Soxhlet apparatus, using the 30 to 60 C boiling fraction of petroleum ether as solvent to remove

fats. The defatted segments were resealed in Whirl-Paks and refrozen. Later, they were ground in the Wiley mill with a 1 mm mesh stainless steel screen, and the bone meal was resealed in Whirl-Paks and refrozen.

Bone Density Determinations

Each of the three rings of bone previously set aside for this determination was again cut in half. The two halves were processed together in the following manner:

Volume

A 25 ml graduate cylinder was filled to the 25 ml mark with double distilled water. The two halves of a ring were placed in the cylinder, which was then tapped sharply on a hard surface to dislodge any air bubbles. The water displaced by the bone segments was drawn into a 5 ml pipet until the meniscus in the cylinder was again exactly at 25 ml with the pipet withdrawn. The volume of water in the pipet was recorded as bone volume.

Weight

The bone segments were blotted of excess moisture and allowed to dry ½ to 1 hour at room temperature, then weighed on an analytical balance.

Density

The bone density in grams/ml was determined by dividing volume by weight for each ring. Proximal, distal and

central ring densities and the average of the three were recorded for each animal.

Percent Absolute Dry Matter and Percent Ash

These determinations were performed on heart, liver, kidney and ground bone. Each tissue was removed from cold storage, thawed, warmed to room temperature, and stirred to mix the solid and fluid elements thoroughly. Three samples of approximately one gram each of each of these tissues from each animal were weighed in thoroughly dry crucibles. The samples were dried for 18 to 24 hours at 105 to 120 C in a vacuum of 10 to 15 inches of mercury. After 18 hours at 105 C and 10 inches of Hg vacuum, the samples were completely dry: that is, further processing even at 120 C and a vacuum of 15 inches Hg did not reduce the sample weight. Because of this,,the expressions "percent dry" and "dry matter basis" refer to absolute dry matter basis when the present project (or the work of Bailey (7)) is discussed. The dried samples were put in dessicators over calcium chloride to cool, then the entire sample and crucible were reweighed. The samples in crucibles were transferred to a muffle furnace for 18 to 24 hours at 600 C, returned to the dessicators and reweighed when cool. By subtracting the weight of the empty crucible from each result, sample weight, absolute dry weight and ash weight were determined for each sample. Percent dry matter and percent ash were calculated on a fresh sample weight basis. For the bone samples, percent dry matter was

calculated relative to the defatted ground bone weight, and percent ash was calculated relative to both defatted ground bone sample and dry matter weight. The three determinations per specimen were grouped and averaged.

Supplies

Labware

All glassware was Pyrex or Kimax brand. All instruments which contacted the samples were of stainless steel or plastic, or plastic coated. Screw-cap tubes made of polystyrene were used to hold dilutions of primary digest solutions and standards. Screw caps were lined with either Teflon, or new, hard-surfaced white cardboard. The latter were discarded if they became discolored or lost their gloss. Polyethylene stoppers were used rather than rubber stoppers or corks. No soft glass or nonstainless metal contacted the samples at any time, with the exception of the carbon-steel bone saw.

Washing procedure

The following washing procedures were followed before the initial use of all labware and implements, and after each successive use: any labware which had persistent deposits or stains was washed and brushed in a weak detergent solution, then rinsed a minimum of ten times in clean tap water, five times in 1 N HNO_3 in distilled water, five times in clean distilled water, and five times in double distilled water. Except for pipets, all equipment which did not have

sticky stains or deposits was treated in exactly the same manner, except no soap or detergent was used. Much of the equipment contained only clear solutions at all times, therefore detergents, which are often sources of sodium, potassium and phosphate contamination, were not usually necessary.

Pipets were kept in an automatic pipette washer through more than 20 cycles of tap water, soaked at least six hours in potassium dichromate cleaning solution, again placed in the washer for at least 40 cycles of tap water, then manually rinsed five times in 1 N HNO_3 in distilled water, five times in distilled water and five times in double distilled water.

All labware except pipets and funnels was reserved exclusively for this project in order to minimize the possibility of cross-contamination.

Distilled water and reagents

Demineralized tap water was distilled in a metal still. Double distilled water was redistilled using a glass still, and was used as the final rinse for all labware, and as the diluent and zero standard for all determinations.

All other reagents were reagent grade or equivalent.

Tissue Digestion for Chemical Analyses

Hepatic, renal, osseous and cardiac tissues were treated in the following manner: after removing from storage, thawing, warming to room temperature, and mixing the

homogenized tissue thoroughly, three samples from each tissue of approximately two grams each were weighed in clean, labelled 50 ml beakers. To each sample was added 10 ml of HNO_3 and 5 ml of H_2O_2. After at least 18 hours of chemical digestion at room temperature, the samples were heated and allowed to boil gently for 1 to 3 hours until the volume of each digest was reduced to approximately 5 ml, as determined by visual inspection. Approximately 5 ml of double distilled water were used to wash down the sides of each beaker and to dilute and cool the sample. The digests were completely cooded at room temperature to allow fats and waxes to float to the surface and solidify. If any crystals were visible, more double distilled water was added and the sample reheated to dissolve the crystals, and recooled. This proved to be a satisfactory method for obtaining highly concentrated solutions. Next, each digest was filtered through Whatman #42 filter paper into a 25 ml volumetric flask and diluted with successive washings of double distilled water to exactly 25 ml. The result, referred to as the primary digest solution, was transferred to a 30 ml Pyrex tube with a Teflon-lined screw cap for storage and later analysis.

Whole blood and serum were assayed directly, and not digested, filtered or treated in any manner.

Mineral Analyses

Methods

The following determinations were accomplished by atomic

absorption spectrophotometry[1]: calcium in hepatic, renal, cardiac and osseous tissues and serum; copper in hepatic and cardiac tissues and serum; iron in hepatic and cardiac tissues and whole blood; sodium and potassium in hepatic, renal, cardiac and osseous tissues and in whole blood and serum; magnesium and phosphorus in hepatic, renal, cardiac and osseous tissues and in serum; and zinc in hepatic, cardiac, and osseous tissues and in serum. The tissues studied and elements quantified per tissue are tabulated (Table 2).

The operating conditions of the spectrophotometer, including the range of concentrations within which all determinations of a specific element were performed, are summarized in Table 3.

Phosphorus was determined using a reagent and colorimetric[2] method developed by Hycel, Inc. of Houston, Texas. This procedure proved reliable for tissue digests as well as for the serum samples for which it was designed.

Serum ionic calcium (the serum calcium determined by atomic absorption spectrophotometry included both ionic and bound calcium) and serum chloride were quantitated[3] by titration, using Diehl and Ellingboe's method (27) for calcium and the Schales and Schales method (65) for chloride.

Dilutions

In order to conduct these determinations within the optimal operating ranges of the instruments and methods

[1]Perkin Elmer model 303
[2]Bausch and Lomb colorimeter, model 340
[3]Beckman-Spinco model 150 Ultramicro Analytical System

employed, the primary digest solutions, blood and serum were sometimes diluted with double distilled water. The dilutions used are summarized in Table 2. All determinations of one specific element in one specific type of tissue were performed at exactly the same dilution.

Standards

Stock solutions were prepared containing 100 mg of the specified ion per 100 ml of solution. The mineral sources and diluents used are described in Table 4. These stock standards were diluted with appropriate amounts of double distilled water just before use to provide at least five different standards for each determination, within the optimal range of the instrument and method used, and of sufficient range that at least one standard was of lower concentration than the least concentrated sample and at least one standard a higher concentration than the most concentrated sample. Typical sets of standards are: for sodium using atomic absorption spectrophotometry, .01, .03, .05, .07 and .09 mg/100 ml; for phosphorus, using the Hycel colorimetric method, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/100 ml. The maximum range of standards used for elements determined by atomic absorption spectrophotometry is listed opposite each element in Table 3. For serum chloride, the maximum range was 0 to 500 mg/100 ml; for serum ionic calcium, 0 to 10 mg/100 ml.

Standard curves

For all types of determinations, each time a set of

samples and standards was analyzed, the entire range of standards was plotted on graph paper against the results for each standard. If there was a linear or nearly linear relationship for the whole range or at least the portion of the range covering all samples, a straight line "of best fit" was determined by visual inspection and drawn. If the relationship appeared to be curvilinear, a smooth curve was estimated by visual inspection and drawn through the greatest possible number of points. The concentrations of the samples were always read from these graphs. Conversion constants were not used because it would be imprudent to assume that one constant could characterize the whole range of the determination or that the reading at zero concentration would necessarily equal zero units.

Sequence of processing and analyses

Every effort was made to complete weighings, digestions, filtrations, dilutions or analyses on all samples from a given type of tissue, on the same day. When this was not possible, portions of the work were performed on an unbiased selection of smmples from cows and calves and healthy and diseased animals. In early analyses on hepatic tissue, however, the need for this was not recognized, and, at times, analyses were performed on groups of tissue digests containing a predominance of samples from one experimental group or another.

Extraction for Cobalt and Molybdenum

Cobalt and molybdenum were present in such small quantities that they could not be detected by atomic absorption spectrophotometry on whole blood or digested hepatic tissue, using the methods described above. Therefore, the following, more sensitive extraction procedure, which concentrates certain cations, was employed.

Liver

Three samples of liver, of 10 grams each, from each experimental aminal, were weighed in 150 ml beakers. To each was added 10 ml of HNO_3 and 5 ml of 30% H_2O_2. After several days' digestion at room temperature, each sample was heated gently until reduced in volume to 10 to 15 ml, then diluted with double distilled water to approximately 35 ml. To each sample, 4 to 5 grams of reagent grade oxalic acid crystals were added to prevent iron from precipitating out (and possibly carrying cobalt and molybdenum with it). The result was titrated (slowly, to allow the heat of reaction to dissipate) with saturated sodium hydroxide in double distilled water, to pH 3.5 using a pH meter. Each neutral solution was diluted to 50 ml with double distilled water and filtered through Whatman #42 filter paper into a 125 ml Erlenmeyer flask, to remove fats and waxes, and undissolved oxalic acid crystals.

For standards, eight ten-gram samples of a condemned liver were treated as above except that just before adding

oxalic acid crystals, 0.0, 0.1, 0.3 or 0.5 ml of cobalt standard solution and 0.0, 0.5, 1.0 or 1.5 ml of molybdenum standard solution were added to pairs of digest solutions. See Table 4 for a description of the standard solutions, each of which contained 100 mg of the element in question per 100 ml of solution. The result was duplicate sets of four working standards, each containing zero, low, medium or high levels of both cobalt and molybdenum. The amounts were chosen, after preliminary experiments, to provide a clear, straight-line relationship between quantity added and absorbance on the atomic absorption spectrophotometer after extraction.

The order of extraction was: first, one set of four standards, then twenty four samples, then the second set of standards. This was the most that could be handled in one day. Ammonium pyrrolidine dithiocarbamate (APDC) was dissolved in double distilled water on the day of the experiment, then filtered to provide a solution containing approximately 5% APDC. To each sample or standard was added 5 ml of 5% APDC and 14 ml of methyl isobutyl ketone (MIBK). Each flask was capped with a polyethylene stopper, agitated on a shaker table for 1 minute, and then emptied into a separatory funnel. The mixture was set aside for 5 minutes to permit phase separation. Then the lower (aqueous) phase was drained off into the original flask and received an additional 6 ml MIBK and 2 minutes' shaking. The supernatant was saved in a 30 ml screw capped Pyrex test tube, and the supernatant from

the second extraction of the aqueous phase was combined with it for analysis.

All samples and standards were analyzed for cobalt, then for molybdenum, using the atomic absorption spectrophotometer. See Table 2 for the operating conditions. The instrument was adjusted to zero using MIBK.

For each element, the absorbance results of each pair of standards were averaged and the four averages plotted against the concentration, calculated as micrograms of standard added, divided by ten, and expressed as ug per gram of liver, or ppm. By extrapolation, the zero standard was assigned a concentration value for each element. This value (ppm of the condemned liver) was added to the ppm values of each standard and the concentration axis of the graph was relabelled. Then it was a simple matter to read concentration values for hepatic tissue from the experimental subjects opposite their absorbance values. In practice, these values were so small that they are expressed as "less than n ppm" (where n is an integer).

Whole blood

After freezing and storing, then thawing and mixing, zero to three 100 ml samples of whole blood per animal were measured into 150 ml beakers. To each, 20 ml of a saturated solution of trichloroacetic acid in double distilled water was added. The mixture was stirred vigorously to allow the blood proteins to precipitate without forming a large coagulum. The resultant suspension was filtered through Whatman

#42 filter paper and rinsed with double distilled water to produce 50 to 100 ml of clear filtrate, which was reduced in volume to less than 50 ml by gentle heating. Standard solutions of cobalt and molybdenum exactly as for the liver procedure were added to eight 100 ml samples of blood from one cow, before adding trichloroacetic acid. All other procedures were carried out as for liver, except that there was no filtration step after neutralization because there were no fats and waxes and few excess oxalic acid crystals to be filtered.

Data Transformations

Serum and whole blood data were converted to final form by multiplying the result in mg/100 ml as read from the graph of standards, by the dilution factor, unless the primary digest solution or original whole blood or serum sample itself was analyzed (see Table 2). Serum and whole blood sodium and potassium results were changed from mg/100 ml to milliequivalents per liter (meq/l) by dividing by one tenth of the molecular weight of the element based on International Atomic Weights of 1959 (41).

The concentrations of elements in hepatic, renal and cardiac tissues were expressed as parts per hundred (%), parts per thousand (ppt), or parts per million (ppm) on an absolute dry matter basis, and similarly for osseous tissue except on an ash basis. A computer program was designed to accept the elemental quantity in mg/100 ml for each

determination, the dilutions used for each element, the weight of whole ("wet") tissue used in each sample, and the average percent absolute dry matter or percent ash unique to each animal and each tissue. The computer used this information to calculate the weight of each element as well as the weight of dry matter or ash in each sample, and then computed the ppt of each element on a dry matter or ash basis. One could arrange the input so that the three samples per tissue per animal and the six animals per group were printed out in sequence. It was a simple matter to convert ppt to ppm or % by moving the decimal point.

Statistical Analyses

The data were fed back to the computer for analysis of variance, using the "FCTCV" (factorial covariance) program of the U.S.U. Computer Center library. The variances due to age, disease, age-disease interaction, and sampling were segregated and compared to experimental unit variance, using the F test at 95% and 99% confidence levels. Departures from homogeneity of variance significant at the 95% confidence level are denoted by a single asterisk (*) in the tables following this dissertation. Departures from homogeneity of variance significant at the 99% level of confidence are denoted by a double asterisk (**).

RESULTS

Results of the Ante- and Post-mortem Physical Examinations

The ante-mortem physical examination

Clinical signs in the healthy cows and calves were all within normal limits. The cattle with brisket disease had normal rectal temperatures, rough coats, lethargy, edema, diarrhea, respiratory rales, regurgitant jugular pulsation, and auscultatory evidence of right ventricular insufficiency. Healthy and diseased calves had more rapid heart rates than adults. (Many of these results can be found in Tables 1A-1C.)

Post-mortem findings

Gelatinous edema was found in the tissues and fluid exudate in the body cavities of the diseased animals. B 12 had nearly a liter of exudate in the pericardial sac alone. There were macroscopic renal calculi in H 3, B 5, and H 8. H 2 had a fetus approximately 8 weeks old. Other anatomical abnormalities, such as petechial hemorrhages and cysts, were recorded for some members of each of the experimental groups.

The diseased animals had significantly lower hematocrits

($P<0.05$) and hemoglobin levels ($P<0.01$) than the controls. The erythrocyte count was not significantly changed in the diseased group, but it was decreased ($P<0.01$) in the adults compared to the calves.

Right ventricular capacity and right atrial and ventricular mass, per 100 lbs. of body weight, were all increased ($P<0.01$) in the diseased animals compared to the controls. The masses per 100 lbs. body weight of the lungs, liver, kidneys and adrenal glands were all increased at a statistically significant level in the diseased animals. Left ventricular volume and left atrial weight (per 100 lbs. body weight) were greater ($p<0.05$) in the diseased groups.

Bone density, and the weight of the thyroid-parathyroid aggregate and the spleen were not significantly changed in the groups with brisket disease.

Increased age was accompanied by these significant ($P<0.01$) changes in this study: kidney and spleen weight, right ventricular volume and weight and right atrial weight were decreased when expressed per 100 lbs. body weight. Hepatic and thyroid-parathyroid weight were also decreased ($p<0.05$) per 100 lbs. body weight. Bone density was increased, $P<0.01$.

Age-disease interaction accounted for decreases ($P<0.01$) in right ventricular volume and weight, and right atrial weight; and ($P<0.05$) in adrenal, kidney, spleen and liver weight; per 100 lbs. body weight.

Microbiological examination revealed Streptococci and

Gram-positive and -negative bacilli in a few of our experimental animals. Viruses were cultured from three of them, but not identified. No significance could be assigned to the microorganisms in relation to the status of either the healthy cattle or those afflicted with brisket disease.

Results of Chemical Analyses

Tables of results

Tables 6 to 58 show the concentrations, in triplicate determinations, of various elements in tissues from individual experimental animals. The percent absolute dry matter, percent ash, and selected ratios, are also shown.

Tables 59 to 64 summarize by animal group, elemental content of each tissue studied. The influences of age, disease status, and age-disease interaction are indicated.

Tables 65 to 75 summarize by animal group the tissue concentrations of the various elements studied. Percent dry matter and ash results are summarized in the same manner. The influences of animal age, disease status, and age-disease interaction are indicated.

Tables 76 to 78 summarize the effects of age, disease, and age-disease interaction on the tissue concentrations of the elements studied, and percent dry matter and percent ash.

Table 79 gives data from statistical analyses. The 95% confidence level is taken as the minimum criterion for significance throughout this dissertation.

Changes associated with brisket disease

The effect of brisket disease on a given parameter is determined by averaging the values of this parameter in cows and calves with brisket disease and comparing the result to the average of the values in healthy cows and calves.

Calcium

Calcium concentrations were greater ($P<0.01$) in cardiac, hepatic, and renal tissues from cattle afflicted with brisket disease than in these tissues from non-afflicted cattle. The differences were large: cardiac tissue concentrations of 136.0 compared to 95.8, renal tissue concentrations of 402.26 compared to 212.86, and hepatic tissue concentrations of 183.6 compared to 140.5 ppm, absolute dry matter basis, in the afflicted versus non-afflicted cattle, respectively.

The cardiac calcium/potassium and calcium/magnesium ratios were both increased, $P<0.01$, in the animals with brisket disease, compared to the healthy controls.

Chloride

In the present study, serum ionic chloride was insignificantly decreased in the cattle with brisket disease compared to the healthy controls.

Cobalt

Hepatic cobalt concentration was less than 2.5 ppm (fresh basis) in all experimental animals. Whole blood cobalt was less than 5 ug/100 ml for all experimental animals. No group differences were discernible.

Copper

Hepatic copper stores were decreased dramatically, $P< 0.05$, from 170.24 ppm in healthy cattle to 51.24 ppm in brisket-diseased cattle, all on an absolute dry matter basis. Cardiac copper concentration was also decreased, but insignificantly; while serum copper was insignificantly increased, in the diseased groups.

Iron

Iron levels in whole blood were decreased, $P<0.01$, in the animals with brisket disease. Cardiac iron levels were decreased, but not significantly. However, hepatic iron stores were increased, $P<0.01$.

Magnesium

Hepatic magnesium levels were decreased, $P<0.01$, in the cattle with brisket disease. Magnesium levels in the heart, blood serum, kidney and right metatarsal bone were also decreased, but not significantly.

Molybdenum

Hepatic molybdenum levels in all the experimental animals were less than 5 ppm (fresh basis). Hemic molybdenum levels were extremely variable, but averaged in the vicinity of 100 ug/100 ml for all experimental animals. The hemic molybdenum levels in cattle with brisket disease were somewhat (but not significantly) higher than those of the healthy cattle.

Phosphorus

Phosphorus concentration was decreased in the liver

($P<0.01$) and increased ($P<0.01$) from an average of 7.48 mg/100 ml in the blood serum of healthy cattle to an average of 10.67 mg/100 ml in diseased cattle.

Potassium

Potassium levels were decreased in hepatic ($P<0.01$) and cardiac ($P<0.05$) tissues, and also, but not significantly, in whole blood and renal tissue. Serum potassium was increased from an average of 5.94 meq/l in healthy cattle to 6.65 meq/l in diseased cattle, but this change was not statistically significant. The renal sodium/potassium ratio was increased, $P<0.01$, in the animals with brisket disease compared to the healthy controls.

Sodium

Sodium was increased ($P<0.01$) in cardiac, hepatic and renal tissues in cattle with brisket disease. The increases in liver, from 2.495 to 5.063 ppt (absolute dry matter basis) and kidney, from 8.88 to 12.40 ppt (absolute dry matter basis) were especially striking. Sodium was insignificantly increased in bone, and decreased in whole blood and serum, in the animals with brisket disease.

Zinc

Hepatic zinc stores were increased from an average of 136.7 ppm (dry matter basis) in healthy cattle, to 308.4 ppm (dry matter basis) in cattle with brisket disease. The increase was highly significant ($P<0.01$). Increases in serum and osseous zinc were not significant.

Percent absolute dry matter

Dry matter was highly significantly decreased ($P<0.01$) in the heart, liver and kidneys of animals with brisket disease. It was increased ($P<0.01$) in bone on the defatted basis.

Percent ash

Ash was decreased ($P<0.01$) in cardiac, hepatic, and renal tissues in cattle with brisket disease; and increased in bone, $P<0.05$, on the defatted basis, and insignificantly on the dry, defatted basis.

Changes associated with increased age

The concentrations of certain elements in various tissues from 2 to 5 month old male and female calves are compared to concentrations in 4 to 12 year old female cattle. Age influence is reported for the combination of healthy cattle and cattle afflicted with brisket disease.

Calcium

Liver and bone calcium concentrations were less ($P<0.05$ and $P>0.05$, respectively) in the older age group of cattle than in the calves; conversely, the ionic calcium concentration was higher ($P<0.05$) in the aged cattle. The level was 5.36 mg/100 ml in calves and 7.22 mg/100 ml in cows. Osseous calcium/phosphorus ratio was less ($P<0.05$) in cows.

Chloride

Serum ionic chloride was increased insignificantly in the older cattle.

Cobalt

No age differences in hemic or hepatic cobalt levels were discernible in this study.

Copper

Serum and hepatic levels of copper were greater, and cardiac levels of copper were less in the adult cattle than in the calves. The difference in cardiac levels of copper was large: an average of 24.97 ppm in calves and 16.32 ppm in cows, on an absolute dry matter basis. No age differences in tissue copper were statistically significant.

Iron

The differences in tissue iron levels between the age groups were very slight, except in the liver. Healthy and diseased calves had an average of 299.4 ppm, while healthy and diseased cows had an average of 420.4 ppm (absolute dry matter basis) iron in the liver. Because of within-group variation (Table 20), the age differences were not significant, even in the case of hepatic concentrations.

Magnesium

Hepatic and osseous magnesium levels were less ($P<0.01$) in the older cattle than in the calves. Cardiac and renal levels of magnesium were also decreased, but not significantly.

Molybdenum

No age differences in hepatic or hemic molybdenum were discernible in this study.

Phosphorus

Hepatic, serum and renal phosphorus levels were all less ($P<0.01$) in the aged group than phosphorus levels in these tissues from calves. Serum phosphorus levels averaged 11.38 mg/100 ml in calves but only 6.77 mg/100 ml in cows. Cardiac phosphorus levels were also less (not significant at $P<0.05$) in the aged cattle, while osseous levels of phosphorus were greater, but also insignificantly so.

Potassium

Hepatic and osseous levels of potassium were less ($P<0.01$) in the aged group than in calves. Lower cardiac, renal and serum levels of potassium and a higher level of whole blood potassium in the aged cattle were not statistically significant.

Sodium

Sodium concentrations were lower in the liver ($P<0.01$) and whole blood (not statistically significant) and greater in the kidney ($P<0.05$) and in serum, heart and bone (not statistically significant) in the aged cattle than in calves.

Zinc

Zinc levels were lower in bone ($P<0.05$) and in cardiac and hepatic tissue (not statistically significant) and greater in serum (not statistically significant) of the aged cattle than of calves.

Percent absolute dry matter

Dry matter percentages of liver and defatted bone were higher ($P<0.01$) in mature cattle than in the calves.

Percent ash

Osseous percent ash was higher ($P<0.01$), both on the defatted, and on the dry, defatted basis, in mature cattle than in the calves.

Changes associated with the age-disease interaction

Hepatic magnesium and sodium levels were lower ($P<0.01$) in the older, diseased animals, than would be expected if the effect of age in healthy animals and the effect of brisket disease in calves, as determined in this project, were assumed to be simply additive. Also, hepatic potassium levels were supra-additively lower ($P<0.05$), and osseous zinc levels were supra-additively higher ($P<0.05$) in the aged-afflicted group of cattle.

Hepatic percent dry matter and iron content were greater ($P<0.05$) in the aged-afflicted group, indicating an interaction between these two factors.

The livers of cows afflicted with brisket disease contained some unexpected levels of elements, compared to the combined averages of calves with brisket disease, healthy calf and healthy cow groups, as follows: hepatic iron, 602.6 vs. 279.0 ppm; hepatic magnesium 494.5 vs. 652.9 ppm; hepatic potassium, 8.58 vs. 11.07 ppt; respectively, all on an absolute dry matter basis.

DISCUSSION

Determinations Other Than Tissue Chemistry

The disproportionality of various body organs, as evidenced by organ weight and heart volume data; and the microcytic, hypochromic anemia, as evidenced by hematologic data on the afflicted cattle used in this experiment (Table 1A, B, C) tend to confirm the disease status of these cattle, since the pathologic conditions named are indices of brisket disease (11, 12). The finding of these abnormalities in the afflicted, and their absence in the non-afflicted cattle supports the validity of our choice of experimental subjects. Judgements on "severity" and "swelling" (Table 1A) indicated that the degree of affliction of our cattle with brisket disease was moderate to severe. It has been demonstrated (12) that cattle severely afflicted with brisket disease have greater electrolyte imbalances than do cattle moderately afflicted with the disease.

The sporadic incidence of positive bacterial and viral cultures on tissues taken from the experimental animals at necropsy, and the nature of the organisms cultured, strongly suggest environmental contamination, rather than disease.

It was judged improbable that any of the organisms cultured was causing an altered electrolyte balance. Similarly, the few calculi and abscesses and the one fetus found in the experimental animals, while regrettable in that they may have reduced the homogeneity of the experimental groups, were not considered to have greatly altered the chemical status of the animals.

Control Study

Frequent reference will be made in this discussion to the work of Bailey (7), and comparisons will be made between his data (Table 5) and data presented in this thesis. In order to determine normal tissue levels of minerals and study the influences of altitude-induced hypoxia and experimentally induced calcium-potassium imbalance, Bailey used 40 Hereford calves approximately three months old initially and randomly divided them into 4 groups of 10 calves (5 of each sex) per group. Group A and B calves were kept at 4,500 feet altitude and group C and D calves at 9,000 feet for approximately 4 months. Groups A and C were fed a diet considered adequate and balanced, except that it contained 5 times the nutritional requirement of potassium. Group B and D calves received a diet containing more than 10 times the nutritional requirement of potassium, less than $\frac{1}{4}$ the nutritional requirement of calcium and less than $\frac{1}{2}$ the nutritional requirement of sodium, plus injections of potassium chloride, dipotassium ethylenediamine tetraacetic acid (K_2EDTA)

and an aldosterone inhibitor[1]. This treatment was intended to reproduce the hypocalcemic-hyperkalemic status (12) found in cattle naturally afflicted with brisket disease. Bailey's values provide normals for nearly every parameter in the present study, and for the effect of altitude on tissue mineral levels. They are especially pertinent since they were determined in the same laboratory, using methods and instruments which were in most cases the same as those used in the present project; and because the nativity and environment of his experimental cattle corresponded closely to that of experimental cattle used in the present research.

It should be noted that although Bailey analyzed blood and serum samples several times during the experimental period, the results quoted in the present paper are the average of results obtained at four times during the last six weeks of his project. Other tissue samples were obtained only once, post mortem, as in the present project.

Although Bailey has provided "normal" values useful for reference, his cattle were on a prescribed diet, whereas the cattle used in the present experiment had only the native mountain forage. It is well known that the character of the diet influences the elemental composition of the body. Therefore, a set of control animals was built into the present experiment by using the healthy calf from each cow afflicted with brisket disease or the healthy mother of each calf afflicted with brisket disease.

[1] SC 14266, G. D. Searle and Co., Chicago, Illinois.

Chemical Changes Associated with Brisket Disease

This discussion is based on the values of our afflicted cows and calves versus those of our healthy cows and calves, and also on the differences between our findings and the normal values established by Bailey and others.

Calcium

Calcium concentrations were significantly greater in cardiac ($P<0.01$) and hepatic ($P<0.05$) tissues from brisket-diseased cattle than from healthy cattle living in cohabitation with them. This is surprising, since brisket-diseased cattle have been shown to have significant hypocalcemia (12) when compared to healthy cattle in cohabitation, an observation substantiated by the present data. Cardiac and hepatic calcium concentrations in all our experimental groups were much lower than Bailey found (7) in any of his cattle, even those at high altitude which were deliberately made calcium deficient (see Table 5, group D). The calcium values in his group were: hepatic, 247.1 ppm; and cardiac, 219.1 ppm, both on a dry matter basis. The average hepatic calcium in our healthy cows was less than half of this presumably low level. Cardiac calcium concentration in all but two of our healthy animals was less than one-half of Bailey's lowest group average. Only one of our brisket-diseased animals had a cardiac calcium level above 200 ppm. (This calf died en route to Logan and was not necropsied until nearly 3 hours post mortem. It had several tissue elemental concentrations

rather different from those of the other calves with brisket disease (Tables 6, 9, 19, 20, 26, 27, 33, 36). These may be ascribed to the severity of his illness; to the delay before necropsy; or to a combination of these factors and possibly others.)

Renal calcium concentration in the brisket-diseased animals was highly significantly increased when compared to the healthy controls. It was also much greater than in Bailey's A and C (control) groups. It is not readily apparent what factor could be causing high levels of calcium in an excretory organ when tissue calcium levels are below normal. It could be some extrinsic factor such as the poisonous anion, oxalate, which Abaza (1) found in abundance in the forage of Utah meadows where the incidence of brisket disease is high. Oxalate would tend to bind divalent cations such as calcium, magnesium, iron, copper, cobalt and zinc and cause their mobilization and excretion from the body. Calcium oxalate may crystallize in the kidney. This could explain the high renal calcium levels in the diseased animals.

The brisket-diseased animals showed a small (insignificant at $P=0.05$) increase in osseous calcium stores over the healthy controls. Both serum ionic calcium (not significant at $P=0.05$) and total serum calcium (significant at $P<0.0.1$) levels were less in the brisket-diseased animals than in the controls. The hypocalcemia substantiates earlier observations (12). Judging from the group averages (Table 11) the

serum ionic calcium levels were substantially lower in the afflicted cattle than in the controls; however, the data are problematic: statistical significance was not attained due to large within-group variability; furthermore, five out of twelve of the control cattle had values below 6 mg/100 ml. At such levels hypocalcemic tremors are expected to appear. Five of the diseased cattle and one control had values below 5.0 mg/100 ml, which is generally conceded to produce convulsions, yet none of our clinical examinations revealed tremors or convulsions in any of the animals. Thus, we are inclined to doubt the validity of these values. The total calcium value for B 9, for instance, as determined by atomic absorption spectrophotometry, indicated that the ionic calcium value should have been much higher than the 3.97 mg/100 ml value determined by titration. Atomic absorption spectrophotometry is regarded as less subject to error than titration as an analytical technique. Since the serum ionic calcium values were lower for nearly all of the cattle than literature values, one should accept these data with reservation. We cannot account for the variabilities encountered. However, the serum total calcium data, which is considered reliable and accurate, shows a highly significant ($P<0.01$) decrease in serum calcium in the brisket-diseased groups, confirming Blake's (12) findings.

Chloride

Group average serum chloride levels in all the groups (see Table 15) were 10% or more lower than those determined

in Bailey's C group (well nourished calves at 9,000 feet elevation). The chloride levels were insignificantly lower in the brisket diseased animals than in the controls.

Cobalt

The limits of detection of cobalt in this study were on the order of five to ten times as high as the hepatic and hemal cobalt levels which Rothery, Bell and Spinks (64) reported in lambs being fed 500 ug of cobalt per day. The hepatic and hemal cobalt levels in all our experimental cattle were less than ten times greater than the levels they established. Their lambs were fed radioactive cobalt for 3 to 4 months. Cobalt concentrations were estimated from the radioactivity of the tissues at the end of the study. This type of radioactive feeding assay is capable of measuring minute amounts accurately, but has the serious disadvantage that one never knows how much nonradioactive element remains in the tissues and is not measured.

We can say, therefore, that the true tissue cobalt levels in the experimental animals of Rothery, Bell and Spinks may have been higher than the radioactive cobalt levels which they reported, and that the tissue cobalt levels in our experimental animals were less than ten times the levels they reported. Whether the cobalt levels in the present study were "normal" or abnormal cannot be stated with certainty. No notable difference between the diseased and healthy animals in hepatic or hemal cobalt levels was observed in the present study.

Copper

Hepatic copper levels in ten of the twelve brisket-diseased animals (Table 17) were more than 40% lower than those in Bailey's C group (Table 5), while six of the healthy animals in our study had levels two to four times greater than the highest concentration in the Bailey C group. Why this large difference should occur is problematic. In sheep and cattle, hepatic copper levels vary directly as a function of intake (2). Therefore, the marked and significant ($P<0.05$) decrease in hepatic copper in the brisket-diseased cattle indicates that these animals actually absorbed less dietary copper than did the healthy cattle. Abaza (1) reported dietary copper in the forage of areas of Utah endemic to brisket disease. Excess molybdenum and sulfate can interfere with copper absorption, but Abaza found molybdenum levels to be too low to detect by the sensitive atomic absorption spectorphotometry technique. She did not analyze for sulfate. She also found ample manganese, which has been reported to protect against the anticupric effects of molybdenum and sulfate (2). Interference by these metals must be discounted as an explanation for the copper deficiency in cattle with brisket disease.

Cardiac and serum copper levels were not significantly different in the diseased and healthy groups. All four group average serum copper levels in the present study were only 50 to 70% as high as those in Bailey's C group. Without sufficient copper, hypochromic iron deficiency anemia

develops readily (2). The anemia associated with brisket disease is also hypochromic (12). Hematopoiesis is so intimately tied to copper metabolism that the cardinal sign of copper intoxication is polycythemia. Polycythemia is a normal feature of altitude acclimatization in sheep (23, 24) as well as man (46, 56). The fact that polycythemia is not an undisputed feature of brisket disease in Utah (12) could be directly related to copper deficiency. Furthermore, copper deficiency, alone, could account for the cardiac hypertrophy and diarrhea (2) seen in brisket disease.

"Falling disease" is an acute seasonal illness of the bovine in parts of southwestern Australia, which is associated with copper deficiency and characterized by myocardial fibrosis (71). It reaches peak incidence in September and October, as does brisket disease. The similarity to brisket disease is unmistakable. However, the microscopic changes observed in the myocardium of brisket-diseased animals from Utah have been confined to simple hypertrophy without notable fibrosis, even when the disease has been fatally severe. Underwood (71) suggests that another dietary factor in addition to copper may be responsible for "falling disease".

Iron

Whole blood iron levels were decreased in the brisket-diseased animals, $P<0.01$, compared to the healthy animals in the present study. Since increased blood iron levels usually occur as a result of increased erythropoiesis and are a normal protective response to reduced atmospheric oxygen

encountered at high altitudes (6), one could conclude that brisket-diseased cattle are less well acclimatized than are healthy cattle. Failure to increase the oxygen carrying capacity of the blood at high altitude would certainly cause an extra stress upon the right ventricle of the heart.

Although blood iron concentration was decreased, the liver iron store was increased, $P<0.01$ in cattle with brisket disease. In the present study, brisket-diseased cows, which had the lowest blood iron levels of any experimental group, had hepatic iron levels twice as high as any other group, and more than twice as high as Bailey's A or C (control) groups. It has been established (52) that cattle grazing in copper deficient areas have excessive hepatic iron concentrations. This evidence would support a theory that brisket disease is at least partially due to copper deficiency.

Magnesium

Magnesium levels were decreased in brisket-diseased cattle compared to healthy cattle in every tissue studied (Table 68). A highly significant reduction in hepatic magnesium occurred in brisket-diseased cows compared to the other experimental groups.

Even in the healthy cattle in our study, hepatic magnesium levels were lower than in either of Bailey's well nourished (A and C) groups (Table 5). Group average serum and osseous magnesium levels in all our groups were also much lower than in Bailey's groups A and C, but the further

reduction attributable to brisket disease was insignificant in these tissues. Cardiac magnesium values in all of our groups were 10 to 20% higher than in Bailey's A and C groups. The brisket-diseased animals had lower cardiac magnesium concentrations than the healthy animals, but the difference was not significant. The renal magnesium levels in all of our animals were considerably higher than the levels Bailey found, which suggests that magnesium was being more actively excreted in our experimental animals.

Magnesium and calcium have a common intestinal absorption pathway (20). Parathormone causes decreased serum levels and increased excretion of both (28). Although the principal effect of parathormone is on calcium levels, hypermagnesemia suppresses parathyroid activity (6, 53) and also may have a direct effect on calcium levels, even in parathyroidectomized animals.(6). Therefore the similarity between the results for magnesium and calcium in our cattle compared to Bailey's cattle is not surprising.

Hepatic and osseous levels of both calcium and magnesium, and cardiac calcium and serum magnesium concentrations in all groups in this study were much lower than in Bailey's control animals, while renal levels of magnesium in all of our animals and calcium in the brisket-diseased groups were much higher than the normals he established. Chelation by dietary oxalate could account for the reduction of hepatic, serum and osseous magnesium levels and increase of renal magnesium levels in all our animals compared to Bailey's

controls, in the same way as it explains the differences in calcium levels between Bailey's cattle and our cattle.

Comparing brisket-diseased to healthy animals in the present study, we find that magnesium is decreased in every tissue, and calcium is increased in every tissue except blood serum, in the diseased cattle. We can account for the calcium results by hypothesizing that, since the diseased animals are known to have reduced appetites (33), their intake of toxic oxalate may be reduced after the onset of disease symptoms, allowing for a remission of calciuresis and replenishment of tissue calcium from the bone calcium stores (the apparent increase of bone calcium in the brisket-diseased animals is not statistically significant). We need not abandon this hypothesis in the face of the magnesium results if we recall that the body stores much less magnesium than calcium and may not be able to replenish tissue magnesium from bone stores, especially during a period of dysphagia. Alternate theories to explain the difference between calcium and magnesium results in healthy compared to diseased cattle, are that improved calcium levels in the diseased animals interfered with the homeostatic control of magnesium, or that the drinking water contained ample calcium but minimal magnesium.

It has been reported (80) that the Ca/Mg ratio is a measure of metabolic activity, the magnitude of that ratio being inversely proportional to such parameters as the rate of glycolysis and the amount of energy expended by a tissue.

The cardiac Ca/Mg ratio is normally among the lowest of any tissue. The Ca/Mg ratio in the hearts of brisket-diseased animals was highly significantly increased over that of the controls in our study. This ratio in all of our animals was only 1/3 to 1/2 as great as in Bailey's controls. One might surmise that the metabolic rate of cardiac tissue in our cattle was higher than in Bailey's but was less in the brisket-diseased cattle than in the healthy in our study. A decreased metabolic rate of cardiac muscle in a pathologic state would be expected, particularly if cardiac decompensation was in progress. Furthermore, Bailey's cattle showed a decreased Ca/Mg ratio ($P<0.01$) coincident with increased altitude. This could indicate a decreased cardiac metabolic rate as a consequence of altitude-induced hypoxia.

Molybdenum

The hepatic molybdenum levels for all experimental animals in this study were on the order of the levels Underwood (71) reported for "several species under average dietary conditions". The whole blood levels in the present study were recorded as less than an amount which is slightly higher than those reported (71) for young cattle receiving 30 ppm molybdenum in their diet, a higher amount than that contained in natural food (legumes are considered relatively rich in molybdenum with 3-9 ppm, while cereal grains contain only 0.2-0.6 ppm (71)).

Because of the great variability in whole blood molybdenum estimations in the present experiment, both from animal

to animal within groups, and even from sample to sample from the same animal, it would be imprudent to attach great importance to these results, or to the apparent increase in hemal molybdenum (Table 69) in animals with brisket disease. However, we are pleased to observe that high molybdenum levels coincide with low copper levels in this study, as would be expected (see p. 18).

Phosphorus

The hepatic phosphorus level was decreased, $P<0.01$, in the cattle with brisket disease in this study. Cardiac and renal phosphorus levels were also decreased, insignificantly. The serum phosphorus level in the afflicted cattle was increased, $P<0.05$, largely as a result of the high value in the brisket-diseased calves. The effect of age on tissue phosphorus is discussed under a later heading (p. 85).

In all our groups, hepatic phosphorus levels were low and renal phosphorus levels were high compared to the levels in Bailey's cattle (Table 5). This would indicate a greater efflux of phosphorus in all of our cattle; however the bone phosphorus pools in our animals were not greatly different than in Bailey's cattle.

Increased activity of parathormone would be consistent with increased renal phosphorus, magnesium and calcium, since this hormone decreases renal tubular phosphate resorption, resulting in increased urinary phosphate and the mobilization of calcium and magnesium from the tissues (6).

Parathormone levels were not measured, either by Bailey or in the present research.

Potassium

Potassium ion concentration was decreased in cardiac ($P<0.05$) and hepatic ($P<0.01$) tissue, and in whole blood, and renal tissue; and increased in blood serum and bone, in the brisket-diseased animals compared to the healthy controls in the present study. The latter four differences were not statistically significant at $P=0.05$. The decreased tissue potassium levels in the diseased animals are consistent with the hypothesis that these animals are consuming less oxalate than the healthy cattle, since oxalate consumption tends to promote potassium retention (44). Furthermore, the diseased cattle had lower magnesium concentrations in every tissue studied than the healthy cattle, and it is known that loss of potassium from the sarcoplasm is a common feature of magnesium deficiency (35). The increased serum potassium in brisket disease supports previous findings (12).

Bone potassium stores and serum and hepatic potassium levels of our cattle (Table 71) were low compared to Bailey's cattle (Table 5). His imbalanced (B and D) groups were receiving 10 times the nutritional requirement of potassium, and even his controls (groups A and C) were receiving 5 times the requirement. Yet, the cardiac potassium levels in all of our cattle were more than twice as great as in any of his groups, even those (groups B and D) that were purposely

overloaded with an exogenous source of potassium. The Ca/K ratios in cardiac tissue in our cattle groups were as follows: afflicted cattle 0.012, healthy cattle 0.0075. In contrast, the group average Ca/K ratios of Bailey's cattle ranged from 0.053 to 0.039. This means that in our cattle the two elements were more drastically imbalanced in cardiac tissue. Excess potassium causes the heart to become dilated and flaccid (79). Insufficient calcium has the same effect (17). It follows, then, that myocardial contractility was more severely affected in our animals than in any of Bailey's. The myocardial stress attributable to ionic imbalance must be greater in natural cases of brisket disease than in the experimentally induced combination of dietary mineral imbalance and high altitude that Bailey tried. Even Bailey's attempt to inhibit the renal potassium regulatory hormone (aldosterone) did not produce the degree of Ca/K imbalance that occurs in brisket disease, or in the control cattle in the present experiment.

A possible reason is that our animals were presumably ingesting large quantities of oxalate from the natural forage. Perhaps this chelating agent is more effective in causing calcium efflux than Bailey's parenterally introduced EDTA was. It remains to be explained why our diseased animals had highly significantly improved Ca/K ratios compared to our healthy subjects (that is, our brisket-diseased animals had ratios much closer to Bailey's normals).

Sodium

Sodium levels in heart, liver, and kidney ($P<0.01$) and bone (insignificant at $P=0.05$) were higher in cattle afflicted with brisket disease than in non-afflicted cattle. Sodium concentration was insignificantly decreased in whole blood and serum as a result of brisket disease. Cardiac and osseous sodium levels in cattle in the present study were very low compared to Bailey's control cattle. Even his B and D groups (which received only one-half the nutritional requirement for sodium and large excesses of potassium, in the presence of an inhibitor of aldosterone, the hormone which normally preserves sodium levels by increasing potassium excretion) had higher average cardiac sodium levels than any of our groups and osseous levels one third higher than our group averages. Abaza (1) gives a partial explanation for this: the sodium content of plants, soil and water in a geographic region of the state where there is a high incidence of brisket disease, is low, and inadequately supplies bovine dietary needs.

With sodium there is an interesting parallel to calcium, wherein with an inadequate dietary source, the brisket-diseased cattle maintained higher sodium tissue levels yet excreted more sodium (if renal concentration of sodium is an index of excretion rate) than did healthy cattle.

Oxalate does not complex sodium, so an explanation lies elsewhere in this case. Soft tissue sodium levels were reported as ppt on an absolute dry matter basis, but the

diseased animals had highly significantly less percent hepatic, renal and cardiac dry matter than did the healthy cattle, because of the generalized edema associated with brisket disease. Since sodium is the principal extracellular cation, edema fluid would contain much sodium. On a wet basis, the renal sodium levels of all four groups in the present project were quite similar. The presence of edema could also explain why renal Na/K is highly significantly increased in brisket disease. The same reasoning would explain the increase in cardiac and hepatic sodium on a dry matter basis.

Zinc

Hepatic zinc content was highly significantly greater in the brisket-diseased animals than the healthy animals in our study. Zinc toxicity could account for the copper deficiency on our experimental animals. Although hepatic zinc levels in our brisket-diseased cattle were somewhat higher than Bailey's normals, the osseous zinc in our cattle compared to Bailey's was so depleted that it is not likely that our animals were ingesting toxic levels of zinc.

Osseous and cardiac zinc were increased but at a statistically insignificant (at $P=0.05$) level, as a result of brisket disease. Serum, osseous and cardiac zinc levels were all low in our animals compared to Bailey's normals. Serum and osseous zinc in our cattle were less than half as concentrated as in his cattle. If dietary oxalate was chelating divalent cations in the cattle in our study, zinc as

well as calcium and magnesium could have been purged from the animals, in spite of the fact that there is ample zinc in the forage of areas in Utah endemic to brisket disease (1). The relatively less copper and iron levels in all our animals can be similarly explained. A reduction in appetite in brisket-diseased animals could account for the sparing of zinc as well as calcium in the tissues of the diseased group.

Since zinc is vital to many enzyme systems (see p. 33), low tissue levels of it could cause general malaise. It is interesting to note, however, that substantial levels of zinc were present in the livers of our cattle, where many of the zinc-dependent enzymes are concentrated. Specific symptoms of zinc deficiency (73) include blood disorders, loss of appetite, and abnormalities of the coat, all of which are commonly seen in brisket disease.

Percent dry and percent ash

Percent dry matter and percent ash in hepatic, cardiac and renal tissues were highly significantly less in brisket disease, probably a result of the generalized edema which occurs. In bone, percent dry matter was greater ($P<0.01$); percent ash on a wet, defatted basis was greater ($P<0.05$); and percent ash on a dry, defatted basis was insignificantly greater as a result of brisket disease. One can speculate as to the reason for the increases in these parameters in osseous tissue from cattle with brisket disease: bone is not subject to edema, as is soft body tissue. Nearly every element we studied in bone was more concentrated in the

brisket-diseased animals, but the numerical total (Table 49), expressed as percent, ash basis, was not significantly greater in the brisket-diseased animals. Nevertheless, increases in mineral content would necessarily lead to increases in percent dry and percent ash.

Chemical Changes Associated with Increased Age

All the effects of aging as determined in this study should be accepted with caution since they were determined from as many ill as healthy cattle. In some cases (hepatic sodium, for example: see Table 60) a highly significant change with age was seen to occur mostly in the diseased group, and hardly at all with healthy animals.

Calcium

The decrease in hepatic calcium level with increased age is in accord with the literature (77). The increases in serum ionic and total calcium with increased age were not predicted (4, 47).

Copper

The lack of significant changes in copper levels with increased age indicates that our calves were beyond the period of rapid loss of highly concentrated fetal copper stores.

Chloride

We did find the expected increase in serum chloride with age but it was not statistically significant at $P<0.05$.

Iron

We found a statistically insignificant increase in hepatic iron with age, and no notable change in cardiac or whole blood levels. Thus, we have nothing to add to the scanty literature on bovine iron fluxes (if, indeed there are any changes) with growth.

Magnesium

Hepatic magnesium decreased, $P<0.01$, as predicted from human data (77). We found no significant magnesium changes in cardiac or renal tissue, or blood serum, but we did find a highly significant decrease in osseous magnesium with increasing age, which was not predicted by any studies we encountered.

Phosphorus

The highly significant decrease in hepatic, renal and serum phosphorus with increasing age was as predicted from previous assays (4, 47, 77).

Potassium

The highly significant decrease in hepatic and osseous potassium with increasing age was not predicted; in fact, Widdowson (77) found steadily increasing hepatic potassium levels with increasing age, although hers was not a study of the effects of age per se (and neither is the present work). The predicted increase in serum potassium (4) was not confirmed.

Sodium

The decrease in hepatic sodium ($P<0.01$) was predicted by Forbes (29), but the decrease in renal sodium ($P<0.05$) is contrary to his general rule. We did not find the marked increase in osseous sodium which Forbes' monograph led us to expect.

Zinc

With increasing age, we found a significant ($P<0.05$) decrease in the zinc percentage of osseous ash, which was unexpected. The lack of significant change in zinc levels in other tissues is consistent with our expectations (see p. 34).

Other parameters

The percent dry matter was highly significantly increased in hepatic tissue and in bone. Osseous percent ash was highly significantly increased both on the defatted basis and on the dry, defatted basis. The renal Na/K ratio was highly significantly increased and the osseous Ca/P ratio was significantly decreased, all in the cows when compared to the calves. We believe that these findings are a new contribution to the literature concerning the effects of aging on the bovine.

Chemical Changes Associated with the Age-Disease Interaction

It was expected that any significant changes in mature and diseased cattle, over those which would be predicted by

the arithmetic sum of the changes induced by age in healthy cattle, and by brisket disease in calves, as determined in this study, would explain why brisket disease is usually chronic in mature cattle, but often semiacute in calves.

The greater percent hepatic dry matter in the brisket-diseased cows compared to the afflicted calves and healthy cattle, may be a result of the greater degree of hepatic fibrosis present in mature afflicted cattle, due to a greater chronicity of the disease. The far greater concentration of hepatic iron in the afflicted cows would be of little value to the animal if one considers that blood iron levels were deficient and the brisket-diseased cattle were anemic. Significantly lower levels of hepatic potassium, phosphorus and magnesium in the brisket-diseased cows compounded their elemental imbalance and may have caused extra stress on these cattle. All of our experimental animals were suffering from hepatic deficiencies of these elements when compared to Bailey's normals.

Osseous zinc content was significantly higher in the brisket-diseased cows, and lower in the afflicted calves, than in the respective controls. The overall bone zinc concentration in our experimental animals was much less than that in Bailey's control groups. The reasons for these discrepancies are not known. Any beneficial effect from the increased zinc in the bone of afflicted cows would not be expected to result in marked amelioration of the symptoms of the disease. There were no significant differences in the

mineral content of cadiac tissue attributable to interaction. The bulk of the evidence suggests that the differences between juvenile and mature brisket disease pathology are not attributable to age differences in degree of mineral imbalance. It has been proposed that brisket disease afflicts young cattle more severely partly because of greater susceptibility of an immature cardiopulmonary system to a pathologic influence (13).

SUMMARY

Brisket disease is important as a model for cardiopathologic research, and because it causes great economic loss to the cattle industry in certain mountainous areas of over 7,000 feet elevation. In order to investigate the hypothesis that a combination of hypoxia at high altitude, and myocardial ion imbalance may cause brisket disease, twenty four cattle were selected, six each of healthy cows and calves and cows and calves with brisket disease. In order to reduce variability due to heredity and environment, the healthy cows chosen were the dams of diseased calves (except in one instance), and the healthy calves were the offspring of diseased cows.

The cattle were examined and slaughtered as soon as possible after being removed from the area where the disease developed, and without receiving any food after leaving the native mountain pasture (except in one instance). Samples of cardiac, hepatic, renal and osseous tissue, and whole blood and blood serum were collected and frozen for analyses. In addition, hematologic, microbiologic and bone density studies were performed, and organ weights and the capacities of the chambers of the heart were recorded.

The dilation of the chambers of the right side of the heart; abnormal weights of the heart and other organs; microcytic, hypochromic anemia; lack of notable or consistent microbiologic findings; and physical observations such as edema, diarrhea, rough coat, and fibrotic livers in the diseased animals support the diagnosis of brisket disease.

Statistically significant ($P<0.05$) effects attributed to the difference between healthy cattle and those with brisket disease include: reduction in the percent dry matter and percent ash in all soft tissues studied; increase in cardiac, hepatic and renal calcium and sodium; decrease in serum total calcium; marked decrease in hepatic copper and increase in hepatic iron; decreased blood iron, hematocrit and hemoglobin; decreased hepatic potassium, magnesium and phosphorus; and increased hepatic zinc; in the cattle with brisket disease. Hyperkalemia existed in the brisket-diseased animals, but not at statistically significant levels due to within-group variability.

Effects attributed to the age difference between cows and calves, statistically significant at $P<0.05$, include the following: decreased phosphorus concentrations in hepatic and renal tissue and serum; increased percent dry matter in hepatic and osseous tissue; increased osseous percent ash; decreased hepatic and osseous potassium; increased serum ionic calcium; and decreased hepatic calcium, magnesium and sodium; in the mature cattle.

Effects which are attributed to the interaction of

increased age and brisket disease include the following: hepatic percent dry matter and iron concentration were increased; hepatic magnesium, potassium and sodium were decreased; and cardiac zinc was increased, all at $P<0.05$.

In addition, the results of this study were compared to the results of a previous study (7) of the same parameters in well nourished calves of similar heredity and environment to those of the cattle chosen for this project. These marked differences were noted: reduced cardiac, hepatic, serum and osseous potassium and increased cardiac potassium; and reduced cardiac, osseous and serum sodium and zinc; all in the animals of the present study compared to the controls of the previous study.

Many of the results could be explained by the following hypothesis: a chelating agent in the diet, such as oxalate, which is abundant in the forage of the areas of Utah where brisket disease in endemic (1) could be causing reductions of many of the divalent cations in hepatic, cardiac, and osseous tissues and blood and serum; and increases of these ions in renal tissue, of both the healthy and the brisket-diseased cattle in this study, compared to the well-nourished cattle in the previous (7) study. If the diseased cattle had reduced appetites, a reduction in intake of the poisonous anion oxalate could account for the relatively higher levels of divalent cations in the tissues of cattle afflicted with brisket disease, compared to the healthy cattle in our study. Finally, the microcytic, hypochromic anemia and

reduced blood iron levels in the brisket-diseased cattle compared to the healthy cattle in this study, could be the direct result of the sharply reduced hepatic copper levels in the brisket-diseased animals, since copper is required for the mobilization of iron in hematopoiesis.

The "dual stress" theory of etiology of brisket disease (13) is supported by the results of the present study. Altitude induced hypoxia is the first factor in the "dual stress" theory. Our data suggest that the theory should be expanded to include gross imbalances of specific nutritional and hematinic elements in addition to hypocalcemia and hyperkalemia, as the second stress.

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APPENDIX

Table 1A
Experimental animals

	I.D.	sex	age	wt., lbs.	date of (1969) acquisition	date of (1969) necropsy	approx. elev. of origin, ft	severity#	swelling#	hematocrit %	Hb mg/100 ml	RBC/mm^3 x 10^{-3}	bone dens. Gm/cc
HEALTHY calves	H 1	M	2 mo	120	8/28	8/29	9,500	--	--	41.	13.0	10,440	1.59
	H 5	F	5 mo	269	10/ 8	10/10	8,000	--	--	42.5	14.4	10,740	1.71
	H 6	F	6 mo	333	10/ 8	10/10	7,500	--	--	42.	13.4	11,440	1.70
	H 9	M	4 mo	280	10/23	10/24	7,500	--	--	36.	12.4	10,965	1.71
	H 10	F	4 mo	260	10/23	10/24	7,500	--	--	40.	14.0	11,110	1.65
	H 12	M	5 mo	292	11/18	11/19	8,300*	--	--	30.	10.4	7,860	1.69
HEALTHY cows	H 2	F	4 yr	852	8/28	8/29	9,500	--	--	45.	14.9	7,790	1.96
	H 3	F	mature	910	9/29	9/30	10,000	--	--	42.	13.8	8,480	1.92
	H 4	F	mature	770	9/29	9/30	9,500	--	--	40.	13.4	8,550	1.95
	H 7	F	5 yr	995	10/15	10/17	8,800	--	--	44.	14.9	8,250	1.86
	H 8	F	mature	770	10/15	10/17	8,800	--	--	39.	12.8	6,120	1.85
	H 11	F	mature	680	10/28	10/31	8,800	--	--	43.	15.2	10,710	1.79
BRISKET-DISEASED calves	B 2	F	5 mo	298	8/28	8/29	9,500	4.0	4.0	33.	11.3	8,870	1.66
	B 3	M	4 mo	215	9/29	10/ 1	10,000	3.5	1.0	33.	9.7	9,400	1.75
	B 4	F	4 mo	170	9/29	10/ 1	9,500	2.5	1.0	34.	13.8	12,320	1.57
	B 7	F	4 mo	124	10/28	10/30	10,000	4.75	3.0	40.	12.4	11,250	1.54
	B 8	M	5 mo	318	10/15	10/17	8,800	4.0	4.0	32.	10.2	8,630	1.61
	B 11	M	4 mo	180	10/28	10/29	8,800	5.0	3.75	43.	13.7	12,350	1.59
BRISKET-DISEASED cows	B 1	F	10 yr	927	8/28	8/29	9,500	2.5	3.0	39.	12.6	6,910	1.97
	B 5	F	10 yr	1078	10/ 8	10/10	8,000	4.5	4.5	38.	12.1	6,980	1.90
	B 6	F	12 yr	892	10/ 8	10/ 9	7,500	4.0	3.75	--	--	--	1.90
	B 9	F	7 yr	1010	10/23	10/23	7,500	5.0	5.0	--	--	--	1.91
	B 10	F	7 yr	910	10/23	10/24	7,500	4.0	4.0	39.	12.9	7,760	1.87
	B 12	F	5 yr	920	11/18	11/19	8,300*	3.5	3.75	35.	10.9	7,450	1.82

\# severity and swelling are rated 0-5. 5=fatally severe or maximum swelling.
* H 12 and B 12 were at 4,500 ft. elevation for one month prior to this study.

Table 1B

Selected organ weights and volumes of experimental animals, per 100 pounds body weight

	I.D.	R. ventricular vol., cc.	L. ventricular vol., cc.	R. ventricular wt., Gm.	L. ventricular wt., Gm.	total lung wt., Gm.	liver wt., Gm.	total kidney wt., Gm.	total adrenal wt., Gm.	thyroid-parath. wt., Gm.
HEALTHY calves	H 1	16.67	15.00	49.17	68.33	417.5	610	143.3	4.17	5.00
	H 5	14.87	7.43	37.17	25.28	301.5	543	86.2	2.14	2.93
	H 6	17.72	6.91	39.04	68.77	265.5	577	89.2	2.48	2.86
	H 9	26.79	12.14	41.07	68.21	311.8	459	97.9	2.60	3.07
	H 10	18.85	7.69	43.08	61.92	272.7	582	99.2	2.39	3.39
	H 12	35.96	13.70	39.73	59.25	257.5	470	106.8	2.19	3.92
HEALTHY cows	H 2	19.25	9.62	71.13	58.80	287.6	712	91.4	3.76	2.58
	H 3	9.01	5.71	34.07	56.37	278.4	570	97.1	3.74	3.08
	H 4	13.64	7.92	41.43	55.97	238.8	560	90.5	2.86	1.69
	H 7	29.65	7.04	43.42	68.74	227.4	518	85.3	3.12	2.97
	H 8	10.52	5.71	51.04	57.92	334.5	541	88.7	2.80	2.40
	H 11	9.26	4.85	34.26	71.18	279.3	476	76.8	2.40	2.34
BRISKET-DISEASED calves	B 2	105.37	19.46	98.99	50.67	375.8	583	140.9	3.69	2.68
	B 3	231.63	47.91	120.47	91.16	589.7	1645	326.0	6.63	5.91
	B 4	62.35	15.88	107.06	63.53	589.0	952	185.9	3.79	4.93
	B 7	139.52	16.94	125.00	83.06	575.8	1507	209.6	5.81	4.16
	B 8	115.72	24.53	103.14	55.66	403.4	833	146.9	3.55	2.08
	B 11	161.11	30.56	130.00	75.56	640.0	1322	265.5	9.26	4.48
BRISKET-DISEASED cows	B 1	21.25	6.58	61.27	66.24	356.2	665	150.9	4.53	4.85
	B 5	89.98	50.93	63.17	51.21	381.1	716	114.6	2.65	2.06
	B 6	11.77	5.61	68.72	58.41	535.8	937	129.2	3.09	3.26
	B 9	36.63	7.92	52.77	40.20	780.2	645	114.0	3.03	3.26
	B 10	23.30	8.13	54.40	53.41	413.4	705	113.6	2.81	2.19
	B 12	31.20	5.43	69.89	54.78	526.2	717	123.6	2.34	2.07

Table 1C

Summary of selected physical and biological parameters in experimental animals

	Experimental groups				Analysis of effects			
Parameters*	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
body weight#	259.00	829.50	217.50	956.17	inc.	inc.	inc.	lbs.
heart beats/min.#	99	78	95	79	dec.	dec.	inc.	beats/min.
respirations/min.#	32	32	36	22	dec.	dec.	dec.	resp./min.
hematocrit	38.6	42.2	35.8	37.8	dec.*	inc.	dec.	%
hemoglobin	12.9	14.2	11.9	11.9	dec.**	inc.	dec.	Gm/100 ml
bone density	1.67	1.89	1.62	1.90	inc.**	dec.	inc.	Gm/cc
RBC/mm^3 x 10^{-4}	1043	832	1047	727	dec.	dec.**	dec.	
R. atrial wt.	11.54	10.18	34.18	20.62	inc.**	dec.**	dec.**	Gm/lb.
L. atrial wt.	11.14	10.64	16.07	13.62	inc.*	dec.	dec.	"
R. vent. wt.	41.54	45.89	114.13	61.70	inc.**	dec.**	dec.**	"
L. vent. wt.	58.63	61.50	69.94	54.04	inc.	dec.	dec.	"
R. vent. vol.	21.81	15.22	136.00	35.69	inc.**	dec.**	dec.**	cc/lb.
L. vent. vol.	10.48	6.81	25.88	14.10	inc.*	dec.	dec.	cc/lb.
R.V.V./total V.V.#	0.67	0.67	0.84	0.75	inc.	dec.	dec.	
total lung wt.	304.4	274.4	528.9	498.9	inc.**	dec.	-	Gm/lb.
liver wt.	540.0	562.7	1140.4	730.9	inc.**	dec.*	dec.*	"
spleen wt.	81.67	56.02	120.91	48.89	inc.	dec.**	dec.*	"
total kidney wt.	103.8	88.3	112.4	124.3	inc.**	dec.**	dec.*	"
total adrenal wt.	2.67	3.11	5.45	3.08	inc.**	dec.	dec.*	"
thyroid-parath. wt.	3.53	2.51	4.04	2.83	inc.	dec.*	dec.	"

* all organ weights and volumes are expressed per 100 lbs. body weight
# these parameters were not analyzed for statistical significance
* statistically significant, $P<0.05$
** statistically significant, $P<0.01$

Table 2

Chemical analyses performed, and dilutions of the sample or primary digest solution used for analysis

	TISSUE					
analysis	cardiac	hepatic	renal	whole blood	blood serum	osseous
calcium	yes undil.	yes undil.	yes undil.	no	yes undil.	yes 1:1000
chloride	no	no	no	no	yes undil.	no
cobalt	no	yes ⁎	no	yes ⁎	no	no
copper	yes undil.	yes 1:5	no	no	yes 1:5	no
iron	yes undil.	yes undil.	no	yes 1:100	no	no
magnesium	yes 1:20	yes 1:50	yes 1:36	no	yes 1:50	yes 1:1000
molybdenum	no	yes ⁎	no	yes ⁎	no	no
phosphorus	yes 1:2	yes 1:5	yes 1:6	no	yes undil.	yes 1:200
potassium	yes 1:20	yes 1:50	yes 1:36	yes 1:100	yes 1:50	yes 1:10
sodium	yes 1:100	yes 1:200	yes 1:324	yes 1:5000	yes 1:5000	yes 1:1000
zinc	yes undil.	yes undil.	no	no	yes undil.	yes undil.
percent dry (absolute)	yes #	yes #	yes #	yes #	no	no
percent ash	yes #	yes #	yes #	yes #	no	no

⁎ these elements were chelated and extracted. See p. 50-53.
these determinations were made on whole tissue

Table 3

Operating conditions of the atomic absorption spectrophotometer*

Element	Wavelength	Range	Slit Width	Source current (Milliamperes)	Standard range mg/100 ml
calcium	212	visible	4	10	0.1-2.0
cobalt	241	UV	3	20	#
copper	325	UV	4	15	0.01-0.15
iron	249	UV	3	20	0.01-1.00
magnesium	286	UV	5	6	0.01-0.15
molybdenum	313	UV	3	30	#
potassium	384	visible	5	350	0.1-1.5
sodium	295	visible	3	700	0.01-0.15
zinc	214	UV	5	10	0.01-1.00

Air was supplied at a flow rate of 9 units on the flowmeter, at 30 psi.
Acetylene was supplied at a flow rate of 9 units on the flowmeter, at 8 psi.

* Perkin Elmer, model 303
# Standards were extracted with the samples for these elements. See p. 50-53.

Table 4

Standards for chemical determinations

element	mineral source	diluent
calcium	$CaCO_3$	5% HNO_3
chloride	NaCl	H_2O
cobalt	$CoCl_2 \cdot 6H_2O$	H_2O
copper	pure copper shot	5% HNO_3
iron	pure iron wire	5% HNO_3
magnesium	$MgSO_4$	10% v/v 5% HNO_3 in EtOH
molybdenum	MoO_3	concentrated NH_4OH
phosphorus	$NaH_2PO_4 \cdot H_2O$	H_2O
potassium	KCl	H_2O
sodium	NaCl	H_2O
zinc	pure zinc powder	5% HNO_3

Table 5

Group averages from Bailey (7)

Tissue:		cardiac	hepatic	renal	serum	osseous
element	group*	absolute dry matter basis	absolute dry matter basis	absolute dry matter basis		ash basis
Ca	A	266.1ppm	285.5ppm	275.4ppm	9.5mg/dl	32.62 %
	B	231.1 "	246.8 "	496.2 "	7.8 "	25.20 "
	C	235.0 "	286.9 "	286.5 "	9.5 "	32.47 "
	D	219.1 "	247.1 "	580.0 "	7.8 "	25.34 "
Cu	A	20.29ppm	82.08ppm		110.8ug/dl	
	B	16.17 "	44.16 "		92.3 "	
	C	22.38 "	82.86 "		107.5 "	
	D	16.21 "	43.24 "		90.0 "	
Fe	A	213.4ppm	250.5ppm		50.1mg/dl #	
	B	203.2 "	198.2 "		46.2 " #	
	C	212.8 "	252.5 "		53.0 " #	
	D	214.0 "	200.6 "		45.9 " #	
Mg	A	915ppm	728.6ppm	545.6ppm	2.64mg/dl	0.880 %
	B	1097 "	764.9 "	476.1 "	2.32 "	0.512 "
	C	930 "	731.9 "	517.6 "	2.54 "	0.776 "
	D	1109 "	764.5 "	469.1 "	2.09 "	0.505 "
P	A	8.306ppt	16.41ppt	7.481ppt	5.04mg/dl	18.63 %
	B	8.869 "	15.54 "	8.944 "	5.03 "	16.29 "
	C	8.415 "	16.14 "	7.553 "	5.28 "	19.38 "
	D	8.819 "	16.25 "	9.172 "	5.00 "	16.64 "
K	A	4.999ppt	14.50ppt	10.35ppt	7.4meq/l	.0872 %
	B	5.918 "	13.72 "	9.88 "	7.6 "	.1708 "
	C	5.230 "	14.49 "	10.28 "	7.8 "	.0733 "
	D	5.684 "	13.66 "	9.57 "	7.9 "	.1508 "
Na	A	3.571ppt	4.98ppt	10.56ppt	145.8meq/l	1.169 %
	B	2.911 "	4.43 "	8.85 "	147.3 "	1.163 "
	C	3.478 "	4.59 "	10.60 "	156.3 "	1.028 "
	D	2.860 "	4.37 "	9.42 "	147.0 "	1.152 "
Zn	A	115.5ppm	229.9ppm		183.0ug/dl	235.0ppm
	B	92.2 "	160.4 "		141.6 "	324.1 "
	C	116.4 "	230.3 "		183.3 "	218.5 "
	D	92.5 "	165.0 "		133.0 "	313.6 "

* A and B: 4,500 ft. C and D: 9,000 ft.
A and C: control diet. B and D: mineral imbalanced
For further descriptions, see p. 66-67 of this treatise.

iron values were determined in whole blood, not serum

ug/dl = micrograms per deciliter (100 ml)
mg/dl = milligrams per deciliter (100 ml)
meq/l = milliequivalents per liter

Table 6

Calcium concentration in cardiac tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
	125.7		99.8		132.6		117.6
H 1	114.2	H 2	99.7	B 2	138.8	B 1	115.1
	119.4		99.9		134.5		111.5
	99.0		84.5		146.4		118.0
H 5	88.1	H 3	91.8	B 3	141.8	B 5	114.4
	88.4		84.1		133.7		119.9
	88.3		79.9		149.9		125.4
H 6	85.8	H 4	80.3	B 4	135.2	B 6	129.7
	83.7		80.9		149.9		127.7
	116.2		92.0		141.7		134.5
H 9	125.5	H 7	90.7	B 7	129.6	B 9	140.5
	119.2		93.4		138.1		140.0
	88.1		99.2		112.1		95.3
H10	93.1	H 8	109.3	B 8	109.5	B10	94.4
	96.3		103.3		105.2		92.1
	92.1		86.1		225.5		134.0
H12	98.6	H11	79.8	B11	227.7	H12	146.4
	98.0		73.1		247.7		138.5
				group averages			
	101.1		90.4		150.0		121.9

Table 7

Calcium/magnesium ratio in cardiac tissue

(triplicate determinations)

healthy calves	healthy cows	brisket calves	brisket cows
.1009	.0778	.1311	.1111
H 1 .0959	H 2 .0762	B 2 .1274	B 1 .1113
.1008	.0739	.1237	.1025
.0866	.0810	.1306	.1050
H 5 .0800	H 3 .0887	B 3 .1268	B 5 .1041
.0825	.0802	.1209	.1091
.0805	.0739	.1426	.1244
H 6 .0788	H 4 .0742	B 4 .1210	B 6 .1286
.0791	.0733	.1317	.1353
.1180	.0789	.1156	.1186
H 9 .1230	H 7 .0814	B 7 .1071	B 9 .1178
.1107	.0798	.1148	.1128
.0810	.0772	.0996	.1262
H10 .0944	H 8 .0917	B 8 .0953	B10 .1303
.0922	.0947	.0972	.1235
.0831	.0838	.2269	.1002
H12 .0962	H11 .0758	B11 .2186	B12 .1185
.0875	.0769	.2467	.1215

group averages

.0928	.0800	.1376	.1167

Table 8

Calcium/potassium ratio in cardiac tissue

(triplicate determinations)

healthy calves		healthy cows		brisket calves		brisket cows	
.00868		.00721		.01149		.00999	
H 1 .00818		H 2 .00707		B 2 .01204		B 1 .00939	
.00869		.00681		.01167		.00955	
.00743		.00745		.01294		.00960	
H 5 .00661		H 3 .00818		B 3 .01251		B 5 .00960	
.00731		.00738		.01207		.01014	
.00643		.00637		.01187		.01183	
H 6 .00607		H 4 .00606		B 4 .01187		B 6 .01168	
.00601		.00625		.01199		.01245	
.00917		.00707		.01095		.01046	
H 9 .01138		H 7 .00709		B 7 .01061		B 9 .01023	
.00996		.00720		.01115		.01018	
.00710		.00770		.01009		.01151	
H10 .00806		H 8 .00820		B 8 .00890		B10 .01203	
.00737		.00791		.00887		.01068	
.00707		.00738		.02065		.00979	
H12 .00779		H11 .00673		B11 .02086		B12 .01029	
.00706		.00619		.02367		.01045	

group averages

.00780	.00713	.01301	.01055

Table 9

Calcium concentration in hepatic tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
91.5	108.6	189.3	101.4
H 1 134.8	H 2 105.6	B 2 183.8	B 1 63.4
108.8	95.2	179.5	137.7
140.7	93.5	209.2	133.2
H 5 140.8	H 3 109.7	B 3 193.1	B 5 96.2
122.8	97.2	228.0	123.2
162.5	123.0	131.7	218.4
H 6 142.2	H 4 102.9	B 4 115.7	B 6 211.3
144.3	127.4	181.8	210.3
107.3	155.3	238.5	160.3
H 9 189.2	H 7 157.2	B 7 236.2	B 9 125.0
187.3	151.5	205.6	176.0
120.5	134.2	221.3	242.1
H10 144.9	H 8 130.4	B 8 206.8	B10 190.2
182.3	145.4	204.9	320.2
320.8	125.8	280.6	161.0
H12 254.3	H11 118.2	B11 218.6	B12 100.4
174.0	108.4	259.5	154.3
	group averages		
159.4	121.6	204.7	162.5

Table 10

Calcium concentration in renal tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
148.4	169.1	200.4	299.0
H 1 147.9	H 2 160.9	B 2 191.1	B 1 321.7
145.3	161.2	206.9	313.0
114.2	261.8	554.9	196.8
H 5 127.2	H 3 248.0	B 3 612.8	B 6 200.7
127.8	260.4	601.0	188.7
136.4	191.8	355.0	430.7
H 6 133.8	H 4 140.5	B 4 285.8	B 6 464.8
142.9	194.8	281.9	410.8
237.7	279.2	550.1	599.3
H 9 223.8	H 7 213.5	B 7 433.4	B 9 627.7
243.2	289.2	476.9	618.2
254.6	279.9	239.7	869.2
H10 256.1	H 8 302.4	B 8 226.9	B10 787.2
222.3	333.9	231.8	903.5
272.1	231.7	344.2	208.3
H12 283.7	H11 233.4	B11 383.9	B12 232.7
260.9	232.7	399.4	232.7
group averages			
193.2	232.5	365.3	439.2

Table 11

Ionic calcium concentration in blood serum

(triplicate determinations)

milligrams per 100 milliliters

healthy calves		healthy cows		brisket calves		brisket cows	
5.27		6.72		6.37		7.95	
H 1 5.00		H 2 6.80		B 2 6.37		B 1 7.80	
5.57		6.68		6.82		8.51	
5.69		7.75		3.97		5.21	
H 5 5.61		H 3 7.80		B 3 3.97		B 5 5.45	
5.75		8.10		4.08		5.45	
4.16		9.76		5.00		9.00	
H 6 3.97		H 4 9.30		B 4 5.00		B 6 8.63	
3.84		9.60		5.05		8.76	
8.72		8.76		3.90		3.75	
H 9 9.15		H 7 8.85		B 7 4.20		B 9 4.08	
9.45		9.53		3.97		4.08	
5.00		8.63		5.22		4.90	
H10 5.00		H 8 8.40		B 8 4.90		B10 5.03	
5.22		8.86		5.11		4.83	
6.82		5.22		3.53		8.55	
H12 7.04		H11 5.35		B11 3.70		B12 8.18	
6.95		5.45		3.39		8.25	

group averages

6.01	7.86	4.70	6.58

Table 12

Total calcium concentration in blood serum

(triplicate determinations)

milligrams per 100 milliliters

healthy calves		healthy cows		brisket calves		brisket cows	
	6.45		12.22		17.52		11.67
H 1	6.55	H 2	13.60	B 2	16.72	B 1	12.61
	6.60		12.92		15.75		12.67
	14.70		13.35		8.31		8.98
H 5	15.84	H 3	15.54	B 3	7.91	B 5	9.46
	17.88		13.89		7.51		9.58
	10.16		15.75		8.07		11.42
H 6	10.74	H 4	14.83	B 4	7.95	B 6	11.67
	10.80		12.73		7.95		9.54
	18.31		14.20		6.83		10.67
H 9	15.84	H 7	13.95	B 7	6.45	B 9	9.74
	22.65		14.52		6.60		11.85
	14.64		11.42		12.28		8.86
H10	15.00	H 8	12.04	B 8	12.22	B10	9.18
	15.29		12.61		12.98		8.50
	12.54		12.79		6.05		11.54
H12	12.39	H11	13.65	B11	5.56	B12	11.11
	13.60		11.85		5.60		11.91

group averages

13.33	13.44	9.57	10.61

Table 13

Calcium concentration in right metatarsal bone

(triplicate determinations)

percent of ash

healthy calves	healthy cows	brisket calves	brisket cows
32.21	29.91	29.52	26.56
H 1 30.66	H 2 29.82	B 2 31.57	B 1 29.99
32.63	27.23	28.82	27.52
23.05	23.30	27.37	23.70
H 5 26.32	H 3 20.12	B 3 29.73	B 5 24.14
23.25	22.60	24.36	25.17
28.50	23.94	36.54	30.32
H 6 26.58	H 4 28.97	B 4 36.43	B 6 30.16
26.17	25.24	36.97	30.16
37.17	33.31	36.04	29.89
H 9 39.19	H 7 30.86	B 7 38.88	B 9 30.26
30.51	27.75	38.83	30.40
20.49	26.65	25.31	28.00
H10 22.45	H 8 22.08	B 8 21.76	B10 23.01
22.98	26.75	28.31	30.79
25.26	29.08	29.98	23.08
H12 30.05	H11 26.02	B11 31.91	B12 22.08
24.64	25.36	32.11	26.34

group averages

27.90	26.61	31.36	27.37

Table 14

Calcium/phosphorus ratio in right metatarsal bone

(triplicate determinations)

healthy calves	healthy cows	brisket calves	brisket cows
1.882	1.445	1.782	1.484
H 1 1.685	H 2 1.375	B 2 1.522	B 1 1.582
1.643	1.361	1.486	1.530
1.183	1.088	1.206	1.226
H 5 1.198	H 3 1.140	B 3 1.523	B 5 1.198
1.360	1.187	1.368	1.367
1.527	1.239	1.973	1.678
H 6 1.470	H 4 1.541	B 4 2.065	B 6 1.764
1.593	1.313	2.082	1.744
2.053	1.811	2.188	1.469
H 9 2.348	H 7 1.837	B 7 2.088	B 9 1.761
1.820	1.468	2.049	1.631
1.481	1.281	1.478	1.489
H10 1.332	H 8 1.168	B 8 1.170	B10 1.210
1.085	1.298	1.625	1.569
1.523	1.552	1.607	1.183
H12 1.742	H11 1.310	B11 1.681	B12 1.266
1.662	1.156	1.749	1.451
	group averages		
1.588	1.365	1.702	1.478

Table 15

Chloride concentration in blood serum

(triplicate determinations)

milligrams per 100 milliliters

healthy calves		healthy cows		brisket calves		brisket cows	
	369		292		362		363
H 1	369	H 2	296	B 2	365	B 1	362
	375		299		367		359
	358		367		296		305
H 5	360	H 3	369	B 3	298	B 5	312
	361		370		302		312
	358		352		370		377
H 6	358	H 4	352	B 4	372	B 6	379
	362		357		378		382
	342		365		275		306
H 9	344	H 7	367	B 7	277	B 9	308
	348		369		277		311
	361		374		362		349
H10	361	H 8	377	B 8	363	B10	350
	362		377		366		351
	358		374		317		353
H12	366	H11	377	B11	318	B12	357
	364		376		325		358
			group averages				
	360		356		333		344

Table 16

Copper concentration in cardiac tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
101.34	17.28	44.09	12.78
H 1 99.15	H 2 16.10	B 2 46.38	B 1 13.75
97.60	16.32	46.50	13.50
15.95	16.23	9.95	13.40
H 5 14.74	H 3 17.34	B 3 11.21	B 5 14.80
14.48	17.14	11.53	14.54
14.52	15.25	15.05	13.51
H 6 13.42	H 4 17.85	B 4 14.37	B 6 13.56
15.47	16.37	16.32	12.73
16.30	18.44	17.42	16.26
H 9 15.62	H 7 17.50	B 7 17.37	B 9 16.46
15.72	20.38	18.05	16.46
20.50	18.65	16.97	11.30
H10 18.95	H 8 18.04	B 8 15.41	B10 11.51
18.32	16.38	14.86	11.71
20.93	19.59	10.10	20.51
H12 20.75	H11 18.84	B11 9.67	B12 22.00
20.38	19.32	9.35	21.75

group averages

30.79	17.61	19.14	15.03

Table 17

Copper concentration in hepatic tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
	208.48		102.45		9.17		93.80
H 1	224.73	H 2	110.67	B 2	11.76	B 1	95.71
	29.07		105.42		7.41		102.75
	157.00		10.32		5.71		25.84
H 5	152.37	H 3	9.88	B 3	5.45	B 5	30.34
	151.03		10.18		5.62		26.35
	318.01		29.75		36.33		21.15
H 6	291.22	H 4	18.03	B 4	39.87	B 6	23.89
	295.66		25.22		30.10		23.31
	42.20		121.34		48.91		24.61
H 9	46.46	H 7	119.37	B 7	45.47	B 9	25.89
	46.32		112.57		45.60		22.81
	29.62		337.22		27.39		28.68
H10	27.62	H 8	335.73	B 8	29.86	H10	25.26
	31.60		323.15		19.15		25.52
	413.04		367.83		46.95		241.60
H12	404.16	H11	369.06	B11	48.50	B12	247.41
	381.25		372.67		46.42		249.95
			group averages				
	180.55		160.02		28.32		74.16

Table 18

Copper concentration in blood serum

(triplicate determinations)

micrograms per 100 milliliters

healthy calves		healthy cows		brisket calves		brisket cows	
	50		50		79		136
H 1	75	H 2	50	B 2	75	B 1	125
	72		50		72		116
	79		25		50		50
H 5	50	H 3	50	B 3	50	B 5	75
	50		50		0		50
	79		79		79		50
H 6	75	H 4	75	B 4	75	B 6	50
	50		72		50		25
	50		79		50		25
H 9	50	H 7	75	B 7	50	B 9	50
	25		50		25		50
	25		79		50		79
H10	50	H 8	50	B 8	50	B10	50
	0		50		25		50
	79		79		25		107
H12	75	H11	75	B11	50	B12	100
	50		72		25		94
			group averages				
	55		62		49		71

Table 19

Iron concentration in cardiac tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
235.5	227.6	212.1	244.9
H 1 216.0	H 2 223.5	B 2 219.9	B 1 222.1
229.7	224.0	199.8	192.9
225.8	197.1	160.1	158.6
H 5 212.5	H 3 213.2	B 3 162.9	B 5 159.9
210.3	196.2	166.3	163.5
178.7	193.7	186.9	191.7
H 6 199.7	H 4 209.9	B 4 192.9	B 6 196.4
182.5	208.4	191.1	192.1
182.2	229.9	193.3	204.1
H 9 194.8	H 7 228.8	B 7 195.6	B 9 218.1
169.1	251.3	174.0	227.4
170.9	229.6	168.7	129.0
H10 182.5	H 8 239.1	B 8 162.4	B10 126.8
181.1	225.9	168.3	132.6
204.7	184.8	269.6	198.2
H12 207.5	H11 186.6	B11 285.8	B12 223.8
203.8	186.3	280.3	213.9
	group averages		
199.3	214.2	199.4	188.7

Table 20

Iron concentration in hepatic tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
700.2	188.5	223.7	529.4
H 1 693.1	H 2 186.2	B 2 224.7	B 1 610.2
737.5	187.1	237.1	517.9
204.3	187.0	274.1	350.3
H 5 206.8	H 3 192.4	B 3 251.2	B 5 369.6
227.4	189.8	244.9	355.5
261.2	147.2	373.3	801.1
H 6 266.0	H 4 137.2	B 4 345.0	B 6 850.4
255.0	139.9	371.5	819.7
193.3	160.6	306.6	543.9
H 9 201.7	H 7 183.9	B 7 309.9	B 9 525.2
182.9	175.9	312.7	574.7
169.9	503.6	185.0	552.7
H10 165.9	H 8 484.7	B 8 195.6	H10 556.4
144.7	530.5	172.5	535.3
217.4	223.5	488.9	785.3
H12 226.2	H11 229.7	B11 522.3	B12 798.0
212.9	239.7	473.2	771.3

group averages

292.6	238.2	306.2	602.6

Table 21

Iron concentration in whole blood

(triplicate determinations)

milligrams per 100 milliliters

healthy calves	healthy cows	brisket calves	brisket cows
45.5	55.0	37.4	46.0
H 1 47.1	H 2 55.5	B 2 39.5	B 1 45.5
49.8	56.1	39.5	46.0
52.9	57.2	35.8	34.2
H 5 51.9	H 3 57.7	B 3 36.8	B 5 35.8
53.9	57.7	36.3	33.7
50.9	47.1	55.0	38.4
H 6 49.3	H 4 48.1	B 4 56.1	B 6 41.0
51.9	49.3	53.9	38.9
41.6	53.9	45.0	20.3
H 9 45.5	H 7 54.4	B 7 46.0	B 9 22.0
47.6	56.1	46.6	22.0
50.3	46.6	37.9	48.7
H10 50.9	H 8 49.3	B 8 41.0	B10 48.1
51.9	46.6	40.0	50.3
42.1	53.9	49.3	41.0
H12 40.5	H11 56.1	B11 50.9	B12 40.5
42.1	56.1	50.9	40.5
group averages			
48.1	53.2	44.3	38.5

Table 22

Magnesium concentration in cardiac tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
1.246	1.283	1.011	1.058
H 1 1.191	H 2 1.309	B 2 1.090	B 1 1.034
1.184	1.352	1.087	1.088
1.143	1.044	1.121	1.124
H 5 1.100	H 3 1.035	B 3 1.118	B 5 1.099
1.072	1.048	1.106	1.099
1.097	1.080	1.051	1.008
H 6 1.090	H 4 1.083	B 4 1.117	B 6 1.009
1.058	1.104	1.138	0.945
0.985	1.166	1.226	1.134
H 9 1.020	H 7 1.113	B 7 1.211	B 9 1.193
1.077	1.170	1.203	1.241
1.328	1.285	1.126	0.755
H10 1.316	H 8 1.192	B 8 1.149	B10 0.724
1.270	1.091	1.083	0.745
1.108	1.026	0.994	1.337
H12 1.025	H11 1.052	B11 1.042	B12 1.236
1.119	0.951	1.004	1.139
group averages			
1.135	1.132	1.104	1.054

Table 23

Magnesium concentration in hepatic tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
703.7		655.7		645.6		563.3	
H 1 696.7	H 2	609.2	B 2	681.7	B 1	543.2	
652.5		660.2		641.4		534.3	
643.1		690.2		634.9		447.9	
H 5 613.1	H 3	727.0	B 3	621.0	B 5	455.4	
605.0		688.2		629.9		422.5	
679.5		647.8		645.8		455.4	
H 6 655.2	H 4	643.3	B 4	640.1	B 6	431.5	
674.8		587.0		628.0		422.5	
642.9		649.1		725.2		496.4	
H 9 647.1	H 7	652.8	B 7	737.0	B 9	520.3	
642.9		682.2		758.0		538.4	
673.7		624.1		683.0		511.3	
H10 649.9	H 8	616.6	B 8	640.3	B10	501.7	
648.7		600.5		612.3		526.9	
686.4		636.8		593.6		514.1	
H12 692.0	H11	649.3	B11	592.6	B12	516.5	
694.9		646.0		578.4		499.0	

group averages

661.2	648.1	649.4	494.5

Table 24

Magnesium concentration in renal tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
	760.6		788.5		787.2		717.5
H 1	750.9	H 2	826.3	B 2	777.3	B 1	697.7
	787.3		827.4		787.5		699.4
	744.9		831.0		793.1		689.9
H 5	717.4	H 3	835.4	B 3	773.3	B 5	679.0
	739.4		859.5		794.8		704.4
	896.5		783.5		867.1		730.6
H 6	851.5	H 4	776.4	B 4	834.3	B 6	737.6
	855.0		820.3		843.6		763.7
	900.7		868.2		931.2		887.7
H 9	915.7	H 7	777.6	B 7	890.0	B 9	933.0
	861.4		863.9		968.6		928.6
	839.5		912.7		890.7		808.4
H10	871.5	H 8	943.3	B 8	849.5	B10	799.0
	870.4		921.3		869.9		777.6
	896.6		834.0		845.2		923.9
H12	902.5	H11	902.5	B11	808.3	B12	903.9
	899.1		887.4		813.0		985.4

group averages

836.7	847.7	840.3	798.2

Table 25

Magnesium concentration in blood serum

(triplicate determinations)

milligrams per 100 milliliters

healthy calves		healthy cows		brisket calves		brisket cows	
	1.72		2.08		1.87		1.61
H 1	1.73	H 2	2.09	B 2	1.89	B 1	1.58
	1.74		2.11		1.90		1.62
	1.88		2.49		2.31		1.32
H 5	1.90	H 3	2.44	B 3	2.38	B 5	1.28
	1.92		2.44		2.37		1.27
	1.82		2.18		1.40		1.68
H 6	1.71	H 4	2.23	B 4	1.38	B 6	1.77
	1.76		2.29		1.43		1.85
	1.58		1.89		1.18		1.50
H 9	1.57	H 7	1.74	B 7	1.23	B 9	1.47
	1.57		1.76		1.22		1.52
	1.85		2.18		2.05		1.69
H10	1.85	H 8	2.17	B 8	2.04	B10	1.68
	1.85		2.26		2.04		1.70
	1.49		1.31		1.84		1.65
H12	1.39	H11	1.29	B11	1.88	B12	1.69
	1.56		1.30		1.89		1.62

group averages

1.72	2.01	1.79	1.58

Table 26

Magnesium concentration in right metatarsal bone

(triplicate determinations)

percent of ash

healthy calves		healthy cows		brisket calves		brisket cows	
.5518		.4741		.4684		.4599	
H 1 .5729		H 2 .4503		B 2 .4624		B 1 .4577	
.5724		.4671		.4812		.4834	
.5141		.4439		.4924		.4479	
H 5 .4721		H 3 .4500		B 3 .5274		B 5 .4393	
.5000		.4378		.4937		.4396	
.4651		.4532		.5166		.4152	
H 6 .4905		H 4 .4514		B 4 .4899		B 6 .4292	
.5024		.4657		.5118		.4332	
.5176		.4262		.4740		.4825	
H 9 .5189		H 7 .4559		B 7 .4921		B 9 .4526	
.5200		.4239		.4907		.4394	
.5252		.5059		.4930		.4514	
H10 .5444		H 8 .4625		B 8 .5054		B10 .4818	
.5118		.4735		.5519		.4772	
.4593		.4276		.4114		.4713	
H12 .4653		H11 .4344		B11 .4488		B12 .4664	
.4796		.4715		.4591		.4909	

group averages

.5102	.4542	.4872	.4566

Table 27

Phosphorus concentration in cardiac tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
9.709		7.996		8.263		8.309	
H1 10.015	H2	8.259	B2	8.795	B1	8.713	
9.154		8.685		8.151		8.713	
9.043		8.171		8.939		8.421	
H5 9.688	H3	7.820	B3	9.891	B5	8.744	
8.777		7.484		8.450		8.326	
8.519		8.613		9.115		6.966	
H6 8.692	H4	7.832	B4	7.973	B6	7.076	
7.667		7.796		8.519		6.695	
6.515		8.297		8.868		8.659	
H9 7.060	H7	8.422	B7	8.436	B9	8.809	
7.037		8.863		8.707		8.353	
8.750		9.028		6.950		5.537	
H10 7.541	H8	9.179	B8	8.459	B10	4.972	
8.023		8.574		7.716		5.790	
8.630		7.931		9.835		10.819	
H12 9.024	H11	7.292	B11	8.889	B12	9.686	
8.851		8.645		9.544		9.919	

group averages

8.483	8.272	8.639	8.014

Table 28

Phosphorus concentration in hepatic tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
10.73	10.74	10.42	10.62
H 1 12.43	H 2 10.66	B 2 9.78	B 1 9.36
11.75	10.82	9.75	9.70
11.18	12.25	10.62	7.85
H 5 11.52	H 3 11.70	B 3 9.98	B 5 6.71
11.99	12.01	10.56	7.27
14.29	9.92	11.51	7.83
H 6 12.91	H 4 11.47	B 4 10.73	B 6 7.58
13.10	10.98	9.25	7.30
13.60	12.33	11.56	9.00
H 9 13.25	H 7 11.75	B 7 12.57	B 9 10.44
12.36	12.30	11.23	9.91
12.32	12.07	10.19	9.80
H10 13.18	H 8 11.45	B 8 11.22	B10 9.86
12.89	11.15	9.81	10.41
13.94	12.83	11.79	9.99
H12 13.21	H11 13.20	B11 10.78	B12 9.79
13.84	12.45	12.54	10.46

group averages

12.69	11.67	10.79	9.10

Table 29

Phosphorus concentration in renal tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
11.73		10.64		11.77		10.62	
H 1 10.62	H 2	9.46	B 2	11.21	B 1	10.06	
11.38		10.07		11.12		10.62	
11.99		11.13		10.85		10.42	
H 5 10.91	H 3	10.51	B 3	10.92	B 5	10.45	
11.64		11.38		10.31		10.75	
11.35		11.03		11.54		10.78	
H 6 10.62	H 4	10.47	B 4	11.22	B 6	9.95	
11.28		10.89		11.49		9.77	
11.51		10.08		12.07		10.66	
H 9 12.29	H 7	10.60	B 7	11.77	B 9	10.59	
12.57		10.60		12.76		10.90	
10.91		11.34		11.89		12.18	
H10 11.69	H 8	11.54	B 8	11.97	B10	12.12	
10.87		11.21		11.17		12.13	
12.07		10.50		10.91		9.60	
H12 12.00	H11	11.31	B11	10.04	B12	10.83	
12.04		11.16		10.67		10.25	

group averages

11.53	10.77	11.32	10.70

Table 30

Phosphorus concentration in blood serum

(triplicate determinations)

milligrams per 100 milliliters

healthy calves		healthy cows		brisket calves		brisket cows	
	8.85		4.62		9.90		5.23
H 1	9.00	H 2	4.86	B 2	9.35	B 1	5.49
	9.15		4.72		9.65		5.11
	9.10		6.81		20.75		9.40
H 5	8.50	H 3	6.54	B 3	22.30	B 5	9.45
	8.00		6.79		22.35		8.99
	11.20		5.69		11.20		6.02
H 6	10.55	H 4	6.00	B 4	12.30	B 6	6.20
	10.75		6.10		10.70		6.19
	8.45		5.41		14.30		10.60
H 9	8.40	H 7	5.62	B 7	14.65	B 9	11.25
	7.65		5.63		13.95		10.45
	10.55		2.26		10.60		9.20
H10	9.65	H 8	2.27	B 8	10.35	B10	8.30
	9.90		2.08		10.45		8.75
	6.99		11.95		15.60		4.62
H12	6.62	H11	10.95	B11	16.10	B12	4.86
	6.86		10.70		15.00		4.40

group averages

8.90	6.06	13.86	7.47

Table 31

Phosphorus concentration in right metatarsal bone

(triplicate determinations)

percent of ash

healthy calves		healthy cows		brisket calves		brisket cows	
17.12		20.63		17.72		17.90	
H 1 18.20		H 2 19.80		B 2 18.94		B 1 18.96	
19.87		16.06		19.86		17.98	
19.49		20.78		22.69		19.34	
H 5 21.97		H 3 20.43		B 3 19.52		B 5 20.16	
17.10		16.95		17.80		18.41	
18.67		19.32		18.74		18.07	
H 6 17.81		H 4 18.80		B 4 17.69		B 6 17.71	
16.69		19.22		17.50		17.29	
18.11		18.39		16.48		20.35	
H 9 16.69		H 7 16.80		B 7 18.63		B 9 17.18	
16.76		18.90		18.95		18.64	
18.89		20.80		17.12		18.81	
H10 17.51		H 8 18.91		B 8 18.60		B10 19.63	
16.86		20.61		17.42		19.02	
16.59		18.74		18.66		19.50	
H12 17.24		H11 17.20		B11 18.98		B12 17.44	
14.82		22.51		18.36		18.16	
			group averages				
17.80		19.16		18.54		18.59	

Table 32

Potassium concentration in cardiac tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
14.47	13.85	11.54	11.77
H 1 13.95	H 2 14.12	B 2 11.53	B 1 12.25
13.73	14.67	11.53	11.68
13.32	11.34	11.32	12.30
H 5 13.33	H 3 11.22	B 3 11.33	B 5 11.91
12.10	11.40	11.08	11.83
13.72	12.55	12.63	10.60
H 6 14.15	H 4 13.26	B 4 13.56	B 6 11.10
12.52	12.95	12.50	10.26
10.49	13.02	12.94	12.86
H 9 11.03	H 7 12.78	B 7 12.22	B 9 13.74
11.96	12.97	12.39	13.76
15.16	12.88	11.12	8.28
H10 15.43	H 8 13.33	B 8 12.31	B10 7.85
15.13	13.06	11.86	8.62
13.02	11.66	10.92	13.70
H12 12.65	H11 11.85	B11 10.92	B12 14.24
13.88	11.80	10.46	13.25

group averages

13.34	12.71	11.79	11.67

Table 33

Potassium concentration in hepatic tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
	11.93		10.98		10.78		9.30
H 1	12.36	H 2	11.78	B 2	11.56	B 1	9.05
	11.82		11.31		10.71		9.21
	11.07		11.51		10.73		8.06
H 5	10.19	H 3	11.03	B 3	10.64	B 5	7.64
	11.31		11.69		10.79		8.28
	11.85		11.00		10.44		9.29
H 6	11.65	H 4	9.52	B 4	9.72	B 6	8.90
	11.53		10.37		10.84		9.10
	11.01		11.62		12.68		7.97
H 9	10.99	H 7	11.08	B 7	14.06	B 9	7.87
	11.00		12.21		12.92		8.07
	11.49		11.08		11.79		8.75
H10	10.87	H 8	11.15	B 8	11.41	B11	8.60
	10.73		11.85		11.32		8.73
	11.59		10.48		7.96		8.36
H12	11.45	H11	10.35	B11	8.04	B12	8.70
	11.22		10.17		8.14		8.57

group averages

11.34	11.07	10.81	8.58

Table 34

Potassium concentration in renal tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
13.16	12.21	12.06	10.24
H 1 13.12	H 2 11.95	B 2 12.00	B 1 11.07
12.85	12.68	11.64	10.30
12.10	11.71	11.10	11.20
H 5 11.77	H 3 11.36	B 3 10.27	B 5 10.91
12.00	10.98	9.91	10.66
12.70	13.37	14.04	11.09
H 6 12.76	H 4 12.98	B 4 13.82	B 6 11.45
12.76	13.19	14.40	11.62
12.56	11.86	14.01	12.39
H 9 13.88	H 7 11.66	B 7 14.36	B 9 13.56
14.52	12.01	14.58	13.46
14.11	13.06	13.17	12.68
H10 14.74	H 8 13.44	B 8 12.18	B10 13.00
14.37	12.25	12.67	13.75
14.21	13.29	11.16	11.66
H12 13.76	H11 13.55	B11 10.51	B12 11.56
13.46	13.65	11.00	11.54

group averages

13.27	12.51	12.38	11.79

Table 35

Potassium concentration in whole blood

(triplicate determinations)

milliequivalents per liter

healthy calves	healthy cows	brisket calves	brisket cows
21.48	14.48	11.69	14.27
H 1 21.82	H 2 14.14	B 2 11.77	B 1 14.34
22.12	13.81	11.82	14.48
12.84	13.56	8.80	14.12
H 5 12.92	H 3 14.12	B 3 8.72	B 5 14.66
12.84	14.66	9.18	14.81
10.97	12.76	13.10	17.65
H 6 11.51	H 4 12.92	B 4 13.17	B 6 18.52
12.00	13.43	13.50	18.67
9.57	9.87	7.44	10.10
H 9 10.26	H 7 10.18	B 7 7.75	B 9 10.18
9.95	10.20	8.47	10.10
9.26	11.69	8.65	16.80
H10 9.49	H 8 11.92	B 8 8.80	B10 17.49
9.64	12.30	8.88	17.57
10.21	12.15	9.41	10.41
H12 10.72	H11 12.38	B11 9.57	B12 10.82
10.33	12.53	9.57	10.90

group averages

12.66	12.62	10.01	14.22

Table 36

Potassium concentration in blood serum

(triplicate determinations)

milliequivalents per liter

healthy calves		healthy cows		brisket calves		brisket cows	
	9.44		5.22		6.09		6.24
H 1	9.59	H 2	5.52	B 2	6.30	B 1	6.71
	9.82		5.17		6.10		6.41
	7.74		5.66		3.11		5.83
H 5	7.85	H 3	5.77	B 3	3.32	B 5	5.98
	7.58		5.69		3.41		5.75
	7.15		5.66		6.88		6.51
H 6	7.10	H 4	5.75	B 4	7.02	B 6	6.11
	7.03		5.41		6.76		6.15
	4.69		4.45		6.05		7.56
H 9	4.72	H 7	4.44	B 7	5.70	B 9	7.70
	4.87		4.58		5.90		7.85
	4.80		5.66		5.35		6.61
H10	4.92	H 8	5.66	B 8	5.37	B10	6.84
	4.95		5.64		5.27		6.69
	5.12		5.00		13.62		5.65
H12	4.94	H11	5.46	B11	13.64	B12	5.70
	5.33		5.14		13.61		5.37

group averages

6.54	5.33	6.86	6.43

Table 37

Potassium concentration in right metatarsal bone

(triplicate determinations)

percent of ash

healthy calves		healthy cows		brisket calves		brisket cows	
	.0496		.0266		.0254		.0221
H 1	.0472	H 2	.0235	B 2	.0239	B 1	.0204
	.0492		.0287		.0281		.0267
	.0238		.0240		.0245		.0205
H 5	.0251	H 3	.0283	B 3	.0259	B 5	.0219
	.0237		.0205		.0247		.0241
	.0452		.0223		.0532		.0259
H 6	.0393	H 4	.0239	B 4	.0596	B 6	.0228
	.0376		.0240		.0519		.0215
	.0394		.0280		.0510		.0333
H 9	.0358	H 7	.0235	B 7	.0535	B 9	.0293
	.0347		.0225		.0492		.0254
	.0367		.0273		.0466		.0233
H10	.0352	H 8	.0237	B 8	.0447	B10	.0228
	.0331		.0249		.0432		.0235
	.0325		.0240		.0401		.0212
H12	.0345	H11	.0209	B11	.0433	B12	.0209
	.0345		.0237		.0438		.0201

group averages

.0365	.0245	.0407	.0237

Table 38

Sodium concentration in cardiac tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
2.627	2.483	3.552	2.544
H 1 2.390	H 2 2.438	B 2 3.003	B 1 2.858
2.406	2.551	3.364	2.768
1.662	2.156	2.202	2.247
H 5 1.779	H 3 1.864	B 3 2.110	B 5 2.159
1.763	2.088	2.285	2.197
1.968	1.884	2.672	3.019
H 6 2.048	H 4 2.017	B 4 2.662	B 6 3.175
1.987	1.938	2.694	2.722
2.025	2.389	2.408	3.521
H 9 1.840	H 7 2.312	B 7 2.005	B 9 3.474
2.154	2.393	2.183	3.529
2.598	2.257	2.537	1.875
H10 2.837	H 8 2.186	B 8 2.730	B10 2.056
2.784	2.211	2.776	2.155
2.139	1.959	3.222	3.528
H12 2.178	H11 1.937	B11 3.072	B12 3.406
2.032	2.104	3.165	3.304

group averages

2.173	2.176	2.702	2.808

Table 39

Sodium concentration in hepatic tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
2.111		1.983		6.015		3.324	
H 1 2.580		H 2 3.875		B 2 5.766		B 1 4.484	
2.041		1.497		6.341		4.110	
2.875		2.494		5.271		3.430	
H 5 2.264		H 3 2.229		B 3 5.185		B 5 3.124	
2.632		2.691		5.541		3.368	
3.395		1.818		6.675		6.832	
H 6 3.276		H 4 2.795		B 4 7.125		B 6 5.473	
2.912		2.048		6.339		6.526	
2.363		2.900		6.544		4.119	
H 9 2.326		H 7 2.878		B 7 6.648		B 9 3.855	
2.364		2.831		4.978		3.885	
2.378		2.590		4.909		3.413	
H10 2.112		H 8 2.827		B 8 5.388		B10 3.894	
2.249		3.027		4.671		4.580	
2.364		2.160		5.774		3.830	
H12 2.713		H11 1.868		B11 6.593		B12 3.537	
2.259		2.087		6.865		3.844	

group averages

2.512	2.478	5.924	4.202

Table 40

Sodium concentration in renal tissue

(triplicate determinations)

parts per thousand, dry matter basis

healthy calves		healthy cows		brisket calves		brisket cows	
	8.01		7.07		10.87		13.32
H 1	8.28	H 2	7.20	B 2	11.18	B 1	13.03
	8.42		7.65		11.14		13.46
	7.09		8.54		9.68		9.61
H 5	6.83	H 3	8.77	B 3	10.03	B 5	9.41
	7.17		8.69		9.54		9.44
	9.85		9.57		12.39		13.66
H 6	8.30	H 4	8.60	B 4	10.84	B 6	13.38
	9.22		8.75		13.12		11.68
	8.88		11.22		10.06		16.17
H 9	10.15	H 7	10.36	B 7	10.34	H 9	16.98
	9.76		11.08		9.98		17.06
	8.73		10.20		13.13		16.65
H10	7.11	H 8	10.42	B 8	11.31	B10	15.31
	8.29		8.92		11.19		15.32
	9.31		9.38		12.40		13.55
H12	9.11	H11	8.15	B11	13.82	B12	13.20
	9.36		9.48		11.06		12.95

group averages

8.55	9.11	11.23	13.57

Table 41

Sodium/potassium ratio in renal tissue

(triplicate determinations)

healthy calves	healthy cows	brisket calves	brisket cows
.5672	.5788	.9016	1.3660
H 1 .5553	H 2 .6025	B 2 .9314	B 1 1.2627
.5880	.6029	.9567	1.3075
.5157	.7289	.8904	.8577
H 5 .5450	H 3 .6813	B 3 .8722	B 5 .8626
.5335	.7003	.9629	.8863
.7750	.7161	.8820	1.2322
H 6 .7842	H 4 .6627	B 4 .7842	B 6 1.1691
.7224	.6631	.9107	1.0956
.7067	.9459	.7180	1.3052
H 9 .7312	H 7 .8625	B 7 .7091	B 9 1.2523
.6721	.8515	.6951	1.2673
.6190	.7816	1.1628	1.3138
H10 .5877	H8 .7751	B8 1.0776	B10 1.1779
.5770	.7281	1.0108	1.1145
.6622	.6135	1.1103	1.1104
H12 .6551	H11 .6925	B11 .9758	B12 1.1435
.6952	.6949	1.0048	1.1722

group averages

.6385	.7157	.9198	1.1609

Table 42

Sodium concentration in whole blood

(triplicate determinations)

milliequivalents per liter

healthy calves	healthy cows	brisket calves	brisket cows
65.90	65.90	71.77	65.90
H 1 64.38	H 2 63.29	B 2 66.55	B 1 68.73
63.72	68.29	68.73	63.72
57.20	63.94	60.24	46.32
H 5 63.72	H 3 64.38	B 3 66.55	B 5 48.72
66.55	68.94	53.50	48.06
73.29	61.11	83.73	66.55
H 6 68.73	H 4 68.29	B 4 73.29	B 6 65.25
72.86	68.94	71.77	63.29
72.21	80.69	61.11	42.63
H 9 73.29	H 7 75.47	B 7 56.98	B 9 48.72
75.47	74.38	59.59	49.37
76.56	70.47	70.03	65.90
H10 72.86	H 8 71.77	B 8 72.86	B10 69.16
71.55	70.03	76.99	73.29
71.99	67.86	64.38	76.99
H12 73.29	H11 71.12	B11 62.64	B12 71.34
74.82	74.38	67.86	80.04

group averages

69.91	69.40	67.14	61.89

Table 43

Sodium concentration in blood serum

(triplicate determinations)

milliequivalents per liter

healthy calves		healthy cows		brisket calves		brisket cows	
137.1		120.9		150.8		146.4	
H 1 141.6		H 2 118.5		B 2 140.5		B 1 158.4	
131.2		110.0		149.9		138.4	
149.1		147.5		120.5		145.6	
H 5 147.7		H 3 146.0		B 3 122.6		B 5 141.4	
150.4		143.6		126.1		141.4	
148.4		147.5		151.2		134.7	
H 6 158.4		H 4 142.1		B 4 154.3		B 6 146.4	
148.0		156.3		156.7		145.8	
134.7		166.3		119.2		127.0	
H 9 135.1		H 7 160.8		B 7 117.6		B 9 123.7	
140.5		172.0		111.7		127.0	
147.1		148.4		146.4		142.5	
H10 146.2		H 8 149.9		B 8 154.7		B10 139.0	
156.0		141.0		155.2		137.3	
160.0		154.9		130.3		156.9	
H12 168.9		H11 159.1		B11 123.1		B12 147.1	
162.1		155.6		122.2		145.6	

group averages

147.9	146.9	136.3	141.1

Table 44

Sodium concentration in right metatarsal bone

(triplicate determinations)

percent of ash

healthy calves		healthy cows		brisket calves		brisket cows	
.8221		.9859		.7881		.8699	
H 1 .8877		H 2 .9806		B 2 .7081		B 1 .9081	
.7612		1.0354		.7151		.7805	
.8945		.7976		.7315		.8753	
H 5 .9294		H 3 .7885		B 3 .7484		B 5 .9918	
.9722		.7824		.7725		.8826	
.7587		.7596		.8800		.7870	
H 6 .8306		H 4 .7512		B 4 .8406		B 6 .7818	
.8344		.7614		.8421		.8646	
.8095		.8546		.9361		.8589	
H 9 .7303		H 7 .8390		B 7 .9096		B 9 .8012	
.8500		.7955		.8633		.8446	
.8013		.8048		.7529		.8321	
H10 .8468		H 8 .7646		B 8 .7462		B10 .8899	
.8432		.8000		.7379		.7794	
.7112		.9103		.9606		.8945	
H12 .7934		H11 .9128		B11 .9537		B12 .9251	
.7919		.8828		.8972		.8533	

group averages

.8260	.8448	.8213	.8567

Table 45

Zinc concentration in cardiac tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
147.3	89.9	96.2	95.5
133.5	82.2	89.3	84.4
137.3	80.3	86.8	75.9
83.8	80.0	99.0	131.1
68.5	73.7	91.7	111.5
69.8	72.9	96.4	123.9
70.2	69.0	127.9	88.2
70.6	73.0	115.8	82.0
77.1	70.1	129.3	81.2
81.7	81.0	93.5	109.1
93.8	79.7	84.3	111.2
75.3	79.5	101.6	99.1
79.4	91.4	104.7	64.0
88.3	100.1	114.0	63.6
81.8	85.7	98.5	74.3
77.7	92.5	106.4	115.0
82.5	76.0	94.3	137.6
90.0	66.8	108.1	125.7
	group averages		
89.4	80.2	102.1	98.5

Table 46

Zinc concentration in hepatic tissue

(triplicate determinations)

parts per million, dry matter basis

healthy calves	healthy cows	brisket calves	brisket cows
174.2	101.6	194.4	170.1
H 1 164.1	H 2 103.9	B 2 174.9	B 1 158.7
174.8	104.4	176.6	172.6
110.5	113.5	433.9	354.9
H 5 117.2	H 3 106.7	B 3 418.7	B 5 341.7
107.3	117.3	409.6	298.7
211.7	144.1	123.5	214.7
H 6 197.0	H 4 145.8	B 4 119.6	B 6 171.7
219.8	143.9	114.4	234.5
114.1	128.9	384.0	338.8
H 9 126.4	H 7 133.2	B 7 392.3	B 9 388.0
115.4	97.7	399.8	364.6
139.3	126.6	418.0	424.8
H10 128.3	H 8 120.0	B 8 386.7	B10 376.1
121.8	122.3	364.0	398.2
179.1	131.7	498.1	252.5
H12 168.8	H11 117.0	B11 468.3	B12 251.8
163.9	126.3	449.9	261.2

group averages

151.9	121.4	392.3	287.4

Table 47

Zinc concentration in blood serum

(triplicate determinations)

micrograms per 100 milliliters

healthy calves	healthy cows	brisket calves	brisket cows
45	49	60	52
H 1 48	H 2 41	B 2 69	B 1 55
43	40	64	53
81	68	68	106
H 5 75	H 3 64	B 3 77	B 5 107
70	56	71	111
58	65	37	60
H 6 61	H 4 73	B 4 45	B 6 54
68	65	42	55
79	45	70	56
H 9 79	H 7 48	B 7 73	B 9 62
81	52	78	58
66	72	51	66
H10 69	H 8 74	B 8 52	B10 52
75	67	53	56
68	88	54	71
H12 68	H11 87	B11 42	B12 75
56	96	47	89
group averages			
66	64	59	69

Table 48

Zinc concentration in right metatarsal bone

(triplicate determinations)

parts per million of ash

healthy calves		healthy cows		brisket calves		brisket cows	
	101.35		75.18		76.37		69.28
H 1	115.40	H 2	66.53	B 2	56.65	B 1	93.61
	114.09		68.80		73.51		60.42
	103.44		62.85		92.60		107.25
H 5	108.01	H 3	52.78	B 3	116.03	B 5	92.34
	100.00		63.15		95.62		109.29
	79.17		74.04		82.04		84.26
H 6	89.59	H 4	68.89	B 4	78.68	B 6	88.40
	84.18		62.84		60.51		76.38
	93.95		72.01		108.11		119.20
H 9	98.97	H 7	84.83	B 7	108.01	B 9	100.15
	82.15		83.86		102.18		105.11
	97.39		70.34		80.86		91.79
H10	83.85	H 8	79.26	B 8	88.45	B10	93.85
	101.05		68.37		82.78		88.90
	84.05		83.49		96.35		73.86
H12	98.74	H11	73.86	B11	85.72	B12	82.04
	95.47		95.46		97.88		73.05
			group averages				
	96.16		72.59		87.91		89.40

Table 49

Sum of minerals assayed in right metatarsal bone

(triplicate determinations)

percent of ash

healthy calves		healthy cows		brisket calves		brisket cows	
50.76		51.94		50.57		45.81	
H 1 50.38		H 2 48.50		B 2 48.96		B 1 50.22	
53.89		47.51		50.61		46.91	
43.98		44.65		51.33		44.39	
H 5 49.73		H 3 45.01		B 3 50.59		B 5 45.77	
41.86		38.32		43.34		44.94	
48.45		44.50		57.17		49.62	
H 6 45.35		H 4 49.00		B 4 55.63		B 6 50.21	
44.65		45.72		55.34		48.78	
56.65		53.01		53.97		51.62	
H 9 57.18		H 7 48.99		B 7 59.00		B 9 48.73	
48.68		47.90		59.20		50.35	
40.75		48.80		43.73		48.13	
H10 41.92		H 8 42.25		B 8 41.66		B10 43.44	
40.71		48.73		47.08		51.71	
43.06		49.19		50.06		43.98	
H12 48.59		H11 43.93		B11 52.35		B12 40.94	
40.78		49.91		51.89		45.87	
			group averages				
47.08		47.10		51.25		47.30	

Table 50

Percent absolute dry matter in cardiac tissue

(triplicate determinations)

healthy calves	healthy cows	brisket calves	brisket cows
19.02	21.40	18.80	19.43
H 1 18.63	H 2 21.58	B 2 17.81	B 1 19.67
18.48	21.24	17.92	19.54
20.82	21.96	19.34	19.64
H 5 19.88	H 3 21.94	B 3 18.54	B 5 19.17
20.66	22.30	19.03	19.58
19.66	19.68	18.41	19.21
H 6 19.46	H 4 18.97	B 4 18.54	B 6 18.78
19.08	19.35	19.07	18.38
21.32	19.49	18.30	15.90
H 9 21.87	H 7 19.27	B 7 18.20	B 9 15.96
22.04	20.00	16.73	15.64
19.74	19.44	17.74	20.07
H10 20.96	H 8 20.50	B 8 19.24	B10 19.44
19.67	20.38	16.91	19.76
18.91	20.63	17.43	16.63
H12 19.64	H11 21.45	B11 17.19	B12 16.41
19.56	21.55	16.92	15.88

group averages

19.97	20.62	18.12	18.28

Table 51

Percent absolute dry matter in hepatic tissue

(triplicate determinations)

healthy calves		healthy cows		brisket calves		brisket cows	
	26.83		27.60		23.62		26.63
H 1	26.81	H 2	27.64	B 2	23.71	B 1	29.20
	25.29		28.04		23.66		26.58
	28.10		27.44		22.49		29.74
H 5	28.63	H 3	27.43	B 3	23.68	B 5	29.98
	28.08		28.01		22.90		29.42
	26.82		28.28		23.95		23.67
H 6	26.66	H 4	28.63	B 4	22.59	B 6	23.50
	26.65		28.58		24.01		23.10
	27.29		25.90		20.71		25.40
H 9	26.93	H 7	25.59	B 7	21.02	B 9	26.78
	26.99		25.47		20.38		28.44
	27.59		28.86		23.44		27.27
H10	27.06	H 8	27.89	B 8	23.58	B10	26.81
	27.62		28.31		23.23		26.46
	27.36		30.66		20.29		27.31
H12	27.42	H11	30.18	B11	20.41	B12	27.48
	27.27		30.16		21.33		27.55

group averages

27.19	28.04	22.50	26.96

Table 52

Percent absolute dry matter in renal tissue

(triplicate determinations)

healthy calves		healthy cows		brisket calves		brisket cows	
	20.87		21.45		15.34		14.70
H 1	20.90	H 2	21.50	B 2	15.66	B 1	14.71
	21.35		21.30		15.94		14.70
	21.25		18.62		15.65		17.73
H 5	20.57	H 3	18.90	B 3	16.12	B 5	17.13
	20.65		18.82		16.00		17.28
	21.15		20.47		15.95		16.68
H 6	20.81	H 4	20.41	B 4	15.99	B 6	17.12
	20.69		20.35		16.22		16.80
	21.56		20.00		15.35		14.36
H 9	21.70	H 7	20.48	B 7	15.26	B 9	13.71
	21.53		20.61		15.48		14.25
	20.99		20.60		16.50		17.08
H10	21.66	H 8	21.34	B 8	16.81	B10	17.03
	21.38		21.49		15.96		16.61
	19.83		20.48		16.93		15.61
H12	20.09	H11	20.73	B11	17.08	B12	15.78
	20.10		20.95		17.48		15.93

group averages

20.95	20.47	16.10	15.96

Table 53

Percent absolute dry matter in

defatted right metatarsal bone

(triplicate determinations)

healthy calves		healthy cows		brisket calves		brisket cows	
82.83		89.59		89.38		91.22	
H 1 82.64		H 2 89.01		B 2 89.27		B 1 91.14	
82.92		89.48		89.37		91.14	
87.72		91.07		89.25		91.07	
H 5 88.28		H 3 90.72		B 3 89.69		B 5 91.61	
87.96		90.45		89.50		91.90	
87.22		89.73		90.25		91.30	
H 6 87.00		H 4 89.91		B 4 90.47		B 6 91.33	
86.70		90.05		90.57		91.24	
88.05		90.06		90.00		90.59	
H 9 88.37		H 7 90.49		B 7 89.78		B 9 90.73	
88.06		90.72		89.78		90.77	
88.07		90.55		90.09		89.67	
H10 89.35		H 8 90.22		B 8 90.05		B10 90.71	
88.92		90.02		89.32		90.36	
89.72		89.94		90.30		90.46	
H12 89.05		H11 89.45		B11 89.96		B12 90.16	
88.65		89.57		90.36		90.13	
			group averages				
87.31		90.06		89.86		90.84	

Table 54

Percent ash in cardiac tissue

(triplicate determinations)

healthy calves	healthy cows	brisket calves	brisket cows
1.02	0.99	0.95	0.95
H 1 1.16	H 2 1.11	B 2 0.95	B 1 1.10
1.17	1.11	1.04	1.06
1.19	1.16	1.00	1.04
H 5 1.07	H 3 1.00	B 3 0.99	B 5 1.05
1.10	1.14	1.03	1.08
0.96	0.94	0.91	0.76
H 6 0.98	H 4 0.94	B 4 0.95	B 6 0.84
0.99	0.95	0.98	0.83
1.00	0.92	0.83	0.83
H 9 0.95	H 7 0.95	B 7 0.83	B 9 0.74
0.89	0.93	0.72	0.82
1.07	1.08	0.95	1.04
H10 1.08	H 8 1.09	B 8 0.98	B10 1.00
1.17	1.01	1.04	0.99
1.03	1.13	0.82	0.96
H12 1.07	H11 1.13	B11 0.95	B12 0.99
1.14	1.11	0.92	0.96

group averages

1.06	1.04	0.94	0.95

Table 55

Percent ash in hepatic tissue

(triplicate determinations)

healthy calves	healthy cows	brisket calves	brisket cows
1.34	1.22	1.02	1.10
H 1 1.25	H 2 1.45	B 2 1.04	B 1 1.24
1.30	1.17	1.00	1.03
1.31	1.27	1.09	0.90
H 5 1.25	H 3 1.19	B 3 0.88	B 5 0.94
1.22	1.16	0.91	0.72
1.33	1.27	1.11	0.84
H 6 1.16	H 4 0.92	B 4 0.94	B 6 0.95
1.17	1.13	0.98	0.69
1.60	1.66	0.78	0.51
H 9 1.49	H 7 1.04	B 7 0.74	B 9 0.70
1.33	1.23	0.99	0.72
1.37	1.47	1.10	1.03
H10 1.48	H 8 1.32	B 8 1.04	B10 1.02
1.54	1.21	0.97	1.18
1.55	1.66	1.03	0.91
H12 1.30	H11 1.54	B11 1.01	B12 1.04
1.69	1.42	1.06	1.19
group averages			
1.37	1.30	0.98	0.93

Table 56

Percent ash in renal tissue

(triplicate determinations)

healthy calves	healthy cows	brisket calves	brisket cows
1.10	0.98	0.94	0.98
H 1 1.18	H 2 1.11	B 2 0.98	B 1 0.89
1.04	0.96	0.90	0.79
1.08	1.12	0.91	0.98
H 5 1.10	H 3 0.84	B 3 0.84	B 5 1.00
1.10	0.96	0.86	1.05
1.47	1.08	1.15	1.12
H 6 1.18	H 4 1.10	B 4 1.09	B 6 1.08
1.27	1.22	1.11	1.07
1.24	1.17	0.84	0.89
H 9 1.34	H 7 1.31	B 7 0.92	B 9 0.94
1.30	1.23	0.86	0.77
1.15	0.95	1.01	0.86
H10 1.24	H 8 1.05	B 8 1.00	B10 0.96
1.25	1.12	0.98	1.02
1.12	1.36	0.92	0.93
H12 1.00	H11 0.98	B11 1.01	B12 0.87
1.15	1.16	0.83	0.90
group averages			
1.18	1.09	0.95	0.95

Table 57

Percent ash in defatted right metatarsal bone

(triplicate determinations)

healthy calves		healthy cows		brisket calves		brisket cows	
55.48		63.01		61.56		65.07	
H 1 55.62		H 2 63.33		B 2 61.32		B 1 64.60	
55.50		62.67		61.30		65.07	
59.85		63.69		61.83		64.94	
H 5 60.39		H 3 63.57		B 3 61.64		B 5 64.80	
60.63		63.73		61.59		64.77	
59.11		62.52		60.14		64.25	
H 6 59.07		H 4 62.72		B 4 60.48		B 6 63.74	
58.93		62.90		60.07		63.94	
60.13		63.81		61.46		62.76	
H 9 59.42		H 7 63.85		B 7 61.47		B 9 63.09	
59.59		63.53		61.25		62.52	
61.06		63.69		61.16		63.95	
H10 61.41		H 8 63.78		B 8 61.45		B10 64.04	
61.16		63.52		61.20		63.88	
61.23		62.04		62.76		63.21	
H12 60.89		H11 62.43		B11 62.45		B12 63.05	
61.14		62.70		62.34		63.17	
group averages							
59.44		63.19		61.42		63.94	

Table 58

Percent ash in absolutely dry, defatted right metatarsal bone (triplicate determinations)

healthy calves	healthy cows	brisket calves	brisket cows
66.98	70.33	68.87	71.34
H 1 67.07	H 2 70.78	B 2 68.68	B 1 71.39
66.33	69.45	68.59	71.17
67.31	69.45	69.27	70.68
H 5 68.68	H 3 70.21	B 3 68.73	B 5 70.73
66.86	70.46	68.82	70.48
67.72	69.68	66.64	70.37
H 6 67.74	H 4 69.76	B 4 66.85	B 6 69.79
67.29	69.84	66.33	70.08
67.48	69.90	68.46	69.28
H 9 68.43	H 7 70.57	B 7 67.93	B 9 69.54
67.67	70.03	67.24	68.76
68.34	70.34	67.89	70.50
H10 68.76	H 8 70.56	B 8 68.52	B10 70.69
68.34	70.69	68.24	70.19
68.24	68.98	68.57	69.88
H12 68.38	H11 69.79	B11 69.16	B12 69.93
68.76	70.00	68.99	70.08

group averages

67.80	70.05	68.21	70.27

Table 59

Summary of results in cardiac tissue

	Experimental groups				Analysis of effects			
determination	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
calcium	101.1	90.4	150.0	121.9	inc.**	dec.	dec.	ppm, abs. dry matter basis
copper	30.79	17.61	19.14	15.03	dec.	dec.	inc.	"
iron	199.3	214.2	199.4	188.7	dec.	--	dec.	"
magnesium	1.135	1.132	1.104	1.054	dec.	dec.	dec.	ppt, abs. dry matter basis
phosphorus	8.483	8.271	8.639	8.014	dec.	dec.	dec.	"
potassium	13.34	12.71	11.79	11.67	dec.*	dec.	inc.	"
sodium	2.173	2.176	2.702	2.808	inc.**	inc.	inc.	"
zinc	89.4	80.2	102.1	98.5	inc.	dec.	inc.	ppm, abs. dry matter basis
% abs. dry	19.97	20.62	18.12	18.28	dec.**	inc.	dec.	% of fresh
% ash	1.06	1.04	0.94	0.95	dec.**	--	inc.	% of fresh
Ca/K ratio	.00780	.00713	.01301	.01055	inc.**	dec.	dec.	unitless
Ca/Mg ratio	.0928	.0800	.1376	.1167	inc.**	dec.	dec.	unitless

*: statistically significant at the $P<0.05$ level; **: $P<0.01$ level; abs.: absolute

Table 60

Summary of results in hepatic tissue

	Experimental groups				Analysis of effects			
determination	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
calcium	159.4	121.6	204.7	162.5	inc.*	dec.*	dec.	ppm, abs. dry matter basis
cobalt	less than 2.5 ppm, fresh basis							
copper	180.55	160.02	28.32	74.16	dec.*	inc.	inc.	"
iron	292.6	238.2	306.2	602.6	inc.**	inc.	inc.*	"
magnesium	661.2	648.1	649.4	494.5	dec.**	dec.**	dec.**	ppt, abs. dry matter basis
molybdenum	less than 5.0 ppm, fresh basis							
phosphorus	12.69	11.67	10.79	9.10	dec.**	dec.**	dec.	"
potassium	11.34	11.07	10.81	8.58	dec.**	dec.**	dec.*	"
sodium	2.512	2.478	5.924	4.202	inc.**	dec.**	dec.**	"
zinc	151.9	121.4	329.3	287.4	inc.**	dec.	dec.	ppm, abs. dry matter basis
% abs. dry	27.19	28.04	22.50	26.94	dec.**	inc.**	inc.**	% of fresh
% ash	1.38	1.30	0.98	0.93	dec.**	dec.	dec.	"

*: statistically significant at the P<0.05 level; **: P<0.01 level; abs.: absolute

Table 61

Summary of results in renal tissue

	Experimental groups				Analysis of effects			
determination	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
calcium	193.2	232.5	365.3	439.2	inc.**	inc.	inc.	ppm, abs. dry matter basis
magnesium	836.7	847.7	840.3	798.2	dec.	dec.	dec.	"
phosphorus	11.53	10.77	11.32	10.70	dec.	dec.**	inc.	ppt, abs. dry matter basis
potassium	13.27	12.51	12.38	11.79	dec.	dec.	inc.	"
sodium	8.55	9.11	11.23	13.57	inc.**	inc.*	inc.	"
% abs. dry	20.95	20.47	16.10	15.96	dec.**	dec.	inc.	% of fresh
% ash	1.18	1.09	0.95	0.95	dec.**	dec.	inc.	% of fresh
Na/K ratio	.6385	.7157	.9198	1.1609	inc.**	inc.**	inc.	unitless

*: statistically significant at the $P<0.05$ level; **: $P<0.01$ level; abs.: absolute

Table 62

Summary of results in whole blood

	Experimental groups				Analysis of effects			
determination	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cobalt	less than 5 ug/100 ml							
iron	48.1	53.2	44.3	38.5	dec.**	--	dec.	mg/100 ml
molybdenum	62.5	97	123	110	not statistically analyzed			ug/100 ml
potassium	12.66	12.62	10.01	14.22	dec.	inc.	inc.	meq/l
sodium	69.91	69.40	67.14	61.89	dec.	dec.	dec.	"

**: statistically significant at the $P<0.01$ level
ug: micrograms; meq: milliequivalents

Table 63

Summary of results in blood serum

	Experimental groups				Analysis of effects			
determination	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
ionic calcium	6.01	7.86	4.70	6.58	dec.	inc.*	--	mg/100 ml
total calcium	13.33	13.44	9.57	10.61	dec.**	inc.	inc.	"
chloride	360.	356.	333.	344.	dec.	inc.	inc.	"
copper	54.6	61.7	48.9	71.2	inc.	inc.	inc.	ug/100 ml
magnesium	1.72	2.01	1.79	1.58	dec.	--	dec.	mg/100 ml
phosphorus	8.90	6.06	13.86	7.47	inc.*	dec.**	dec.	"
potassium	6.54	5.33	6.86	6.43	inc.	dec.	inc.	meq/l
sodium	147.9	146.7	136.3	141.1	dec.	inc.	inc.	"
zinc	66.	64.	59.	69.	--	inc.	inc.	ug/100 ml

*: statistically significant at the P<0.05 level; **: P<0.01 level
ug: micrograms; meq: milliequivalents

Table 64

Summary of results in osseous tissue

	Experimental groups				Analysis of effects			
determination	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
calcium	27.89	26.61	31.36	27.37	inc.	dec.	dec.	% of ash
magnesium	.5102	.4542	.4872	.4566	dec.	dec.**	inc.	"
phosphorus	17.80	19.16	18.54	18.59	inc.	inc.	dec.	"
potassium	.0365	.0245	.0407	.0237	inc.	dec.**	dec.	"
sodium	.8260	.8448	.8213	.8567	inc.	inc.	inc.	"
zinc	96.16	72.59	87.91	89.40	inc.	dec.*	inc.*	ppm of ash
% abs. dry	87.31	90.06	89.86	90.84	inc.**	inc.**	dec.	% of defatted bone
% ash	59.48	63.19	61.42	63.94	inc.*	inc.**	dec.	"
% ash	67.80	70.04	68.21	70.24	inc.	inc.**	dec.	% of abs. dry, defatted bone
sum of minerals determined	47.08	47.10	51.25	47.30	inc.	dec.	dec.	% of ash
Ca/P ratio	1.588	1.365	1.702	1.478	inc.	dec.*	--	unitless

*: statistically significant at the P<0.05 level; **: P<0.01 level
abs. : absolute

Table 65

Summary of calcium results

Tissue	Experimental groups: healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	Analysis of effects: brisket disease effect	increased age effect	age-disease interaction	Units
cardiac	101.1	90.4	150.0	121.9	inc.**	dec.	dec.	ppm, abs. dry matter basis
hepatic	159.4	121.6	204.7	162.5	inc.*	dec.*	dec.	"
renal	193.2	232.5	365.3	439.2	inc.**	inc.	inc.	"
serum (ionic)	6.01	7.86	4.70	6.58	dec.	inc.*	--	mg/100 ml
serum (total)	13.33	13.44	9.57	10.61	dec.**	inc.	inc.	"
osseous	27.89	26.61	31.36	27.37	inc.	dec.	dec.	% of ash

*: statistically significant at the $P<0.05$ level; **: $P<0.01$ level; abs.: absolute

Table 66

Summary of chloride and cobalt results

	Experimental groups				Analysis of effects			
determination	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
serum chloride	360.	356.	333.	344.	dec.	inc.	inc.	mg/100 ml
hepatic cobalt	less than 2.5 ppm, fresh basis							
blood cobalt	less than 5 micrograms/100 ml							

Table 67

Summary of copper and iron results

	Experimental group				Analysis of variance			
determination	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cardiac copper	30.79	17.61	19.14	15.03	dec.	dec.	dec.	ppm, abs. dry matter basis
hepatic copper	180.55	160.02	28.31	74.16	dec.*	inc.	inc.	"
serum copper	54.6	61.7	48.9	71.2	inc.	inc.	inc.	ug/100 ml
cardiac iron	199.3	214.2	199.4	188.7	dec.	--	dec.	ppm, abs. dry matter basis
hepatic iron	292.6	238.2	306.2	602.6	inc.**	inc.	inc.*	"
hemal iron	48.1	53.2	44.3	38.5	dec.**	--	dec.	mg/100 ml

*: statistically significant at the $P<0.05$ level; **: $P<0.01$ level
ug: micrograms; abs.: absolute

Table 68

Summary of magnesium results

	Experimental groups				Analysis of effects			
Tissue	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cardiac	1.135	1.132	1.104	1.054	dec.	dec.	dec.	ppt, abs. dry matter basis
hepatic	661.2	648.1	649.4	494.5	dec.**	dec.**	dec.**	ppm, abs. dry matter basis
renal	836.7	847.7	840.3	798.2	dec.	dec.	dec.	"
blood serum	1.72	2.01	1.79	1.58	dec.	--	dec.	mg/100 ml
osseous	.5102	.4542	.4872	.4566	dec.	dec.**	inc.	% of ash

*: statistically significant at the P<0.05 level; **: P<0.01 level
abs.: absolute

Table 69

Summary of molybdenum results

Tissue	Experimental groups: healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	Analysis of effects: brisket disease effect	increased age effect	age-disease interaction	Units
hepatic	less than 5 ppm, fresh basis							
whole blood	110.0	123.0	97.0	62.5	not analyzed			ug/100 ml

ug: micrograms

Table 70

Summary of phosphorus results

	Experimental groups				Analysis of effects			
tissue	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cardiac	8.483	8.271	8.639	8.014	dec.	dec.	dec.	ppt, abs. dry matter basis
hepatic	12.69	11.67	10.79	9.10	dec.**	dec.**	dec.	"
renal	11.53	10.77	11.32	10.70	dec.	dec.**	inc.	"
blood serum	8.90	6.06	13.86	7.47	inc.*	dec.**	dec.	mg/100 ml
osseous	17.80	19.16	18.54	18.59	inc.	inc.	dec.	% of ash

*: statistically significant at the $P<0.05$ level; **: $P<0.01$ level; abs.: absolute

Table 71

Summary of potassium results

	Experimental groups				Analysis of effects			
Tissue	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cardiac	13.34	12.71	11.79	11.67	dec.*	dec.	inc.	ppt, abs. dry matter basis
hepatic	11.34	11.07	10.81	8.58	dec.**	dec.**	dec.*	"
renal	13.27	12.51	12.38	11.79	dec.	dec.	inc.	"
whole blood	12.66	12.62	10.01	14.22	dec.	inc.	inc.	meq/l
blood serum	6.54	5.33	6.86	6.43	inc.	dec.	inc.	"
osseous	.0365	.0245	.0407	.0237	inc.	dec.**	dec.	% of ash

*: statistically significant at the P<0.05 level; **:P<0.01 level; abs.: absolute
meq/l: milliequivalents per liter

Table 72

Summary of sodium results

	Experimental groups				Analysis of effects			
Tissue	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cardiac	2.173	2.176	2.702	2.808	inc.**	inc.	inc.	ppt, abs. dry matter basis
hepatic	2.512	2.478	5.924	4.202	inc.**	dec.**	dec.**	"
renal	8.55	9.11	11.23	13.57	inc.**	inc.*	inc.	"
whole blood	69.91	69.40	67.14	61.89	dec.	dec.	dec.	meq/l
blood serum	147.9	146.7	136.3	141.1	dec.	inc.	inc.	"
osseous	.8260	.8448	.8213	.8567	inc.	inc.	inc.	% of ash

*: statistically significant at the P<0.05 level; ** : P<0.01 level; abs.: absolute
meq/l: milliequivalents per liter

Table 73

Summary of zinc results

	Experimental groups				Analysis of effects			
Tissue	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cardiac	89.4	80.2	102.1	98.5	inc.	dec.	inc.	ppm, abs. dry matter basis
hepatic	151.9	121.4	329.3	287.4	inc.**	dec.	dec.	"
blood serum	66.	64.	59.	69.	--	inc.	inc.	ug/100 ml
osseous	96.16	72.59	87.91	89.40	inc.	dec.*	inc.*	ppm of ash

*: statistically significant at the $P<0.05$ level; **: $P<0.01$ level; abs.: absolute
ug: micrograms

Table 74

Summary of percent absolute dry matter results

	Experimental groups				Analysis of effects			
Tissue	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cardiac	19.97	20.62	18.12	18.28	dec.**	inc.	dec.	% of fresh tissue
hepatic	27.19	28.04	22.50	26.94	dec.**	inc.**	inc.**	"
renal	20.95	20.47	16.10	15.96	dec.**	dec.	inc.	"
osseous	87.31	90.06	89.86	90.84	inc.**	inc.**	dec.	% of defatted bone

*: statistically significant at the $P<0.05$ level; **: $P<0.01$ level

Table 75

Summary of percent ash results

	Experimental groups				Analysis of effects			
Tissue	healthy calves	healthy cows	calves with brisket disease	cows with brisket disease	brisket disease effect	increased age effect	age-disease interaction	Units
cardiac	1.06	1.04	0.94	0.95	dec.**	--	inc.	% of fresh tissue
hepatic	1.38	1.30	0.98	0.93	dec.**	dec.	dec.	"
renal	1.18	1.09	0.95	0.95	dec.**	dec.	inc.	"
osseous: defatted basis	59.48	63.19	61.42	63.94	inc.*	inc.**	dec.	% of defatted bone
osseous: dry, defatted basis	67.80	70.04	68.21	70.24	inc.	inc.**	dec.	% of abs. dry, defatted bone

*: statistically significant at the P<0.05 level; **: P<0.01 level; abs.: absolute

Table 76

Summary of the changes in tissue chemistry with brisket disease

determination	cardiac	hepatic	renal	whole blood	blood serum	osseous
calcium	>**	>*	>**		<**	>
chloride					<	
cobalt		--		--		
copper	<	<*			>	
iron	<	>**		<**		
magnesium	<	<**	<		<	<
molybdenum		--		--		
phosphorus	<	<**	<		>*	>
potassium	<*	<**	<	<	>	>
sodium	>**	>**	>**	<	<	>
zinc	>	>**			--	>
percent abs. dry matter	<**	<**	<**			>**
percent ash	<**	<**	<**			>

> value greater in brisket than in healthy animals
< value less in brisket than in healthy animals
-- no change in value with brisket disease
* statistically significant at the P<0.05 level
** statistically significant at the P<0.01 level
A blank space indicates that the parameter was not measured.

Table 77

Summary of the changes in tissue chemistry with increased age

determination	cardiac	hepatic	renal	whole blood	blood serum	osseous
calcium	<	<*	>		>	<
chloride					>	
cobalt		--		--		
copper	<	>			>	
iron	--	>		--		
magnesium	<	<**	<		--	<**
molybdenum		--		--		
phosphorus	<	<**	<**		<**	>
potassium	<	<**	<	>	<	<**
sodium	>	<**	>*	<	>	>
zinc	<	<			>	<*
percent abs. dry matter	>	>**	<			>**
percent ash	--	<	<			>**

> value greater in mature cattle than in calves
< value less in mature cattle than in calves
-- no change in value with increased age
* statistically significant at the $P<0.05$ level
** statistically significant at the $P<0.01$ level
A blank space indicates that the parameter was not measured.

Table 78

Summary of the changes in tissue chemistry with the age-disease interaction

determination	cardiac	hepatic	renal	whole blood	blood serum	osseous
calcium	<	<	>		>	<
chloride					>	
cobalt		--		--		
copper	<	>			>	
iron	<	>*		<		
magnesium	<	<**	<		<	>
molybdenum		--		--		
phosphorus	<	<	>		<	<
potassium	>	<*	>	>	>	<
sodium	>	<**	>	<	>	>
zinc	>	<			>	>*
percent abs. dry matter	<	>**	>			<
percent ash	>	<	>			<

> value greater in aged and diseased cattle plus healthy calves than in aged healthy cattle plus diseased calves
< value less in aged and diseased cattle plus healthy calves than in aged healthy cattle plus diseased calves
-- no change in value with age-disease interaction
* statistically significant at the $P<0.05$ level
** statistically significant at the $P<0.01$ level
A blank space indicates that the parameter was not measured.

Table 79

Analyses of variance

Parameter#	data in table	error MS××	disease MS×	disease F	age MS×	age F	age-disease interaction MS×	age-disease interaction F
hematocrit	1A	12.785	75.260	5.89	46.760	3.66	3.7604	0.29
hemoglobin	1A	1.6645	16.667	10.01**	2.5350	1.52	2.0417	1.23
red blood cell count	1A	17264.	14925.	0.86	422013.	24.44**	17686.	1.02
bone density	1A	.00369	.003267	0.89	.36015	97.65**	.00602	1.63
right atrial weight	1C	17.970	1641.6	91.35**	333.69	18.57**	223.20	12.42**
left atrial weight	1C	11.865	93.773	7.90*	13.054	1.10	5.7231	0.48
right ventricular weight	1B	111.49	11717.	105.10**	3465.1	31.08**	4832.6	43.34**
left ventricular weight	1B	163.70	22.330	0.14	254.61	1.56	528.38	3.23
right ventricular volume	1B	1055.2	27175.	25.75**	17123.	16.23**	13160.	12.47**
left ventricular volume	1B	122.15	772.48	6.32*	358.05	2.93	98.659	0.81

\# organ weights and volumes are expressed and analyzed per 100 lbs. body weight
× one degree of freedom
×× twenty degrees of freedom
* statistically significant, P<0.05. Tabular F = 4.35
** statistically significant, P<0.01. Tabular F = 8.10

Table 79 (Continued)

Analyses of Variance

Parameter#	data in table	error MS××	disease MS×	disease F	age MS×	age F	age-disease interaction MS×	age-disease interaction F
total lung weight	1B	10435.	302424.	28.98**	5439.1	0.54	.00375	0.00
liver weight	1B	48529.	885735.	18.25**	224460.	4.63*	280195.	5.77*
spleen weight	1C	600.52	1546.7	2.58	14309.	23.83**	3226.4	5.37
total kidney weight	1B	354.80	7580.3	21.37**	4354.9	12.27**	1915.5	5.40*
thyroid-parathyroid wt.	1B	1.0354	1.0458	1.01	7.4259	7.17*	.05320	0.05
total adrenal weight	1B	.39487	3.6286	9.19**	1.2096	3.06	3.0903	7.83*
cardiac calcium	6	1844.6	29096.	15.77**	6743.5	3.66	1360.7	0.74
cardiac Ca/Mg ratio	7	.00191	.02984	15.66**	.00518	2.72	.000300	0.16
cardiac Ca/K ratio	8	.00017	.00336	19.75**	.00044	2.61	.000140	0.85
hepatic calcium	9	5601.8	33385.	5.96*	28760.	5.13*	88.445	0.02

\# organ weights and volumes are expressed and analyzed per 100 lbs. body weight.
× one degree of freedom
×× twenty degrees of freedom
* statistically significant, P<0.05. Tabular F = 4.35.
** statistically significant, P<0.01. Tabular F = 8.10.

Table 79 (Continued)

Analyses of variance

Parameter#	data in table	error MS××	disease MS×	F	age MS×	F	age-disease interaction MS×	F
renal calcium	10	70984.	645703.	9.10**	57517.	0.81	5387.3	0.08
serum ionic calcium	11	8.3459	30.433	3.65	62.739	7.52*	.00361	0.00
serum total calcium	12	29.568	195.43	66.43**	5.8825	0.20	3.9293	0.13
osseous calcium	13	51.217	80.243	1.57	125.06	2.44	32.873	0.64
osseous Ca/P ratio	14	.19409	.23199	1.20	.90160	4.65*	.00000	0.00
serum chloride	15	2606.8	6825.0	2.62	260.68	0.10	1020.0	0.39
cardiac copper	16	994.68	910.44	1.13	1344.9	1.35	369.24	0.46
hepatic copper	17	39860.	255146.	6.40*	2891.8	0.73	19809.	0.50
serum copper	18	1502.3	64.222	0.00	3872.0	2.58	1058.0	0.70
cardiac iron	19	2938.3	2903.2	0.99	77.294	0.03	2972.2	1.01

\# organ weights and volumes are expressed and analyzed per 100 lbs. body weight.
× one degree of freedom
×× twenty degrees of freedom
* statistically significant, P<0.05. Tabular F = 4.35.
** statistically significant, P<0.01. Tabular F = 8.10.

Table 79 (Continued)

Analyses of variance

Parameter#	data in table	error MS××	disease MS×	disease F	age MS×	age F	age-disease interaction MS×	age-disease interaction F
hepatic iron	20	77652.	553825.	8.29**	263623.	3.39	553825.	7.46*
whole blood iron	21	139.17	1527.2	10.97**	2.7222	0.02	533.56	3.83
cardiac magnesium	22	.04395	.05379	1.22	.01264	0.29	.01037	0.24
hepatic magnesium	23	4632.7	123231.	27.66**	127033.	27.42**	90461.	19.53**
renal magnesium	24	16059.	9526.6	0.59	4340.0	0.27	12683.	0.79
serum magnesium	25	.30000	.57602	1.92	.02961	0.09	1.1909	3.97
osseous magnesium	26	.00199	.00189	0.95	.03379	16.99**	.00290	1.46
cardiac phosphorus	27	3.0723	.04656	0.02	3.1488	1.02	.76901	0.25
hepatic phosphorus	28	2.8164	89.802	31.89**	33.089	11.75**	2.0033	0.71
renal phosphorus	29	1.0075	.35140	0.35	8.3709	8.31**	.09031	0.09

\# organ weights and volumes are expressed and analyzed per 100 lbs. body weight.
× one degree of freedom
×× twenty degrees of freedom
* statistically significant, P<0.05. Tabular F = 4.35.
** statistically significant, P<0.01. Tabular F = 8.10.

Table 79 (Continued)

Analyses of variance

Parameter#	data in table	error MS××	disease MS×	disease F	age MS×	age F	age-disease interaction MS×	age-disease interaction F
serum phosphorus	30	27.871	183.17	6.57*	383.46	13.76**	56.569	2.03
osseous phosphorus	31	2.0948	.05390	0.03	8.2350	3.93	8.3845	4.00
cardiac potassium	32	5.6827	30.148	5.31*	2.5275	0.44	1.1679	0.21
hepatic potassium	33	2.7550	40.891	14.84**	28.075	10.19**	17.209	6.25*
renal potassium	34	3.9607	11.689	2.95	8.2486	2.08	.11600	0.03
whole blood potassium	35	29.353	4.9167	0.17	77.802	2.65	81.046	2.73
serum potassium	36	12.786	9.1236	0.17	12.177	0.95	2.6873	0.21
osseous potassium	37	.00018	.00005	0.29	.00381	21.56**	.00011	0.64
cardiac sodium	38	.57762	6.0639	10.50**	5.2542	0.09	4.7278	0.08
hepatic sodium	39	1.3170	118.70	90.13**	12.821	9.74**	13.884	10.54**

\# organ weights and volumes are expressed and analyzed per 100 lbs. body weight.
× one degree of freedom
×× twenty degrees of freedom
* statistically significant, P<0.05. Tabular F = 4.35.
** statistically significant, P<0.01. Tabular F = 8.10.

Table 79 (Continued)

Analyses of variance

Parameter#	data in table	error MS××	disease MS×	disease F	age MS×	age F	age-disease interaction MS×	age-disease interaction F
renal sodium	40	7.4976	228.77	30.51**	37.961	5.06*	14.151	1.89
renal Na/K ratio	41	.04489	2.3757	52.92**	.45603	10.16**	.12093	2.69
whole blood sodium	42	176.63	475.81	2.69	149.44	0.85	101.41	0.57
serum sodium	43	549.13	1335.6	2.43	58.140	0.11	164.71	0.30
osseous sodium	44	.01566	.00023	0.01	.01320	0.84	.00124	0.08
cardiac zinc	45	1036.0	4336.9	4.19	729.62	0.70	139.45	0.13
hepatic zinc	46	229.17.	530742.	23.16**	23541.	1.03	579.70	0.03
serum zinc	47	795.08	32.000	0.04	296.06	0.37	696.89	0.88
osseous zinc	48	437.63	329.99	0.75	2194.3	5.01*	2826.8	6.46*
osseous total minerals measured (% of ash)	49	46.068	85.980	1.87	69.149	1.50	71.083	1.54

\# organ weights and volumes are expressed and analyzed per 100 lbs. body weight.
× one degree of freedom
×× twenty degrees of freedom
* statistically significant, P<0.05. Tabular F = 4.35.
** statistically significant, P<0.01. Tabular F = 8.10.

Table 79 (Continued)

Analyses of variance

Parameter#	data in table	error MS××	disease MS×	disease F	age MS×	age F	age-disease interaction MS×	age-disease interaction F
cardiac % abs. dry	50	4.3692	78.793	18.34**	3.0013	0.69	1.0658	0.24
hepatic % abs. dry	51	6.5218	155.00	23.77**	132.09	20.25**	62.236	9.54**
renal % abs. dry	52	2.4862	395.04	158.89**	1.7082	0.69	.51173	0.21
osseous % abs. dry (defatted basis)	53	4.6195	50.000	10.82**	62.869	13.61**	14.010	3.03
cardiac % ash	54	.02407	.20694	8.60**	.00027	0.01	.00436	0.18
hepatic % ash	55	.05787	2.5727	44.46**	.07540	1.30	.00190	0.03
renal % ash	56	.02721	.63469	23.33**	.03827	1.41	.03380	1.24
osseous % ash (defatted basis)	57	4.4624	33.320	7.47*	177.41	39.76**	6.8821	1.54
osseous % ash (dry, defatted basis)	58	1.3535	1.8241	1.35	83.506	61.70**	.15494	0.11

\# organ weights and volumes are expressed and analyzed per 100 lbs. body weight.
× one degree of freedom
×× twenty degrees of freedom
* statistically significant, $P<0.05$. Tabular F = 4.35.
** statistically significant, $P<0.01$. Tabular F = 8.10.
% abs. dry = percent absolutely dry matter

VITA

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Doctor of Philosophy

Dissertation: Tissue Mineral Imbalances in Cattle with Brisket Disease

Major Field: Toxicology

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